Some physical, chemical and biopharmaceutical studies on salicylsalicylic acid.

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SOME PHYSICAL, CHEMICAL AND BIOPHARMACEUTICAL STUDIES ON SALICYLSALICYLIC ACID

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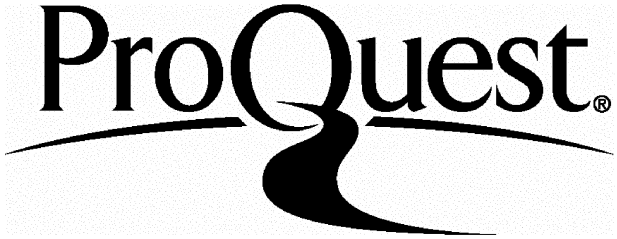

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SUMMARY

This thesis describes some of the physical, chemical and biopharmaceutical properties of a poorly soluble salicylate, salicylsalicylic acid (SSA). In the introduction influences on drug absorption from the gastro-intestinal tract are described. These influences are of physical, chemical, physiological and pharmaceutical origin and are discussed with respect to drugs that are known to exhibit a poor or variable absorption that is limited by their solubility and dissolution behaviour. Methods for improving gastro-intestinal absorption of such drugs are introduced and the advantages of their coprecipitate formation with polyvinylpyrrolidone (PVP) and various bile acids, cholic (CA) and deoxycholic (DOCA) are reviewed. Salicylsalicylic acid is presented as a drug suggested to show dissolution rate-limited absorption and was considered as a suitable model for investigating the influences of various pharmaceutical and formulatory manoeuvres on its absorption.

The experimental sections are divided according to *in vitro* and *in vivo* disciplines.

The *in vitro* studies describe the characterization of SSA with respect to purity, particle size distribution and surface area, crystallinity, melting point, solubility, pKa, partition coefficient, stability, dissolution rate and the influence of different media on its recrystallisation. These studies showed the drug to exhibit poor solubility and high stability in acidic media and established a relationship between particle size and dissolution rate. Parameters relevant to the pH-partition hypothesis indicated that, provided SSA

was in solution, then its absorption would occur throughout the entire gastro-intestinal tract. The preparation of SSA coprecipitates and admixtures with PVP, CA and DOCA are described together with their subsequent characterisation for purity, crystallinity and, in the case of the SSA:CA systems, their *in vitro* dissolution.

In vivo studies are preceded by an introduction to some basic pharmacokinetic concepts and the methods of pharmacokinetic analyses used in this thesis. Investigations were performed in man to establish a correlation between plasma and saliva salicylate levels following oral doses of SSA. This correlation was not shown and, following a report of the limited studies carried out in man, the majority of this section deals with studies in the rat. Various pharmacokinetic criteria were established between the dose of SSA given by intravenous and oral routes. The drug was formulated as a suspension and the influences of suspending agent and particle size were ascertained. A relationship between *in vitro* dissolution rate and *in vivo* absorption for material of varying particle sizes was established and, in an attempt to improve bioavailability, the absorption of SSA from coprecipitates and admixtures with PVP, CA and DOCA was determined. These formulatory manoeuvres met with varied success; indeed, only the SSA:CA 1:1 systems significantly improved drug availability. These studies are discussed with respect to the literature and suggestions for further work are presented.

CONTENTS

CHAPTER ONE

INTRODUCTION

In order to exert a pharmacological effect a drug has generally to attain a certain concentration at the receptor site. Above this minimum concentration the intensity of the pharmacological response is a function of that concentration at the receptor site, the precise measurement of which is generally impossible due to the unknown or diffuse location of the receptor or its inaccessability. The drug is rarely, if ever, administered directly to the receptors but, is made available to the body at a site remote from the site of action, reaching the receptor via the various fluids of distribution. The concentration of the drug in the blood or other body fluids (C.S.F., urine, synovial fluid, etc) is used inferentially to gain knowledge of the concentration at the receptor site and is correlated, wherever possible, with direct physical measurements, e.g. muscle contraction, heart rate, urine flow, or a pharmacological response. It is assumed that an equilibrium exists between the drug concentration in the blood and other body tissues and organs such that a change in blood concentration reflects a change at the receptor site. Frequently therefore, the therapeutic efficiency of a drug formulation, i.e. its onset, duration and intensity of action, is stated in terms of drug concentration in the blood. This concentration is a function of the absorption, distribution, metabolism and excretion characteristics of the drug which are in turn determined by the physical and chemical properties of the drug molecule and the nature of the biological systems involved.

Most drugs are administered by the oral route and in order to elicit any systemic effect they have to cross the gastrointestinal barrier to reach the blood. The rate at which this occurs varies widely in different portions of the gastrointestinal tract because of the

environmental differences encountered in the different regions in terms of pH, surface area, peristalsis, enzyme concentrations, fluid content, etc. These physiological influences on drug absorption following oral administration are well documented and are summarised in Table 1.1. Many extensive reviews on the subject have been published (Schanker, 1971; Prescott, 1975; Rowland, 1973; Gibaldi, 1971; Bates and Gibaldi, 1970).

However, it is believed that the mechanism of absorption is the same in all parts of the gastrointestinal tract and, wherever a drug of structure alien to the body has to cross a biological membrane, it is believed to do so by a process of passive diffusion. Very simply, the membrane may be considered as a bimolecular lipid layer surrounded by protein, although localised variations in this structure are probable in order to account for specific functions. The drug, in order to cross the membrane, has therefore to partition from aqueous solution into the lipid layer and then move out again on the other side into solution in the aqueous medium. The driving force is the concentration gradient of the absorbable form of the drug in solution on either side of the membrane.

The rate of absorption is a complex function of many interrelated biological, physical and chemical characteristics, although much information regarding the absorptive process can be gained from a consideration of the kinetics of diffusion.

Table 1.1 Physiological Influences on Gastrointestinal Drug Absorption.

PROPERTIES OF LUMENAL FLUIDS	
	pH
	mucus interactions
	complexing components
	surface activity
	bile complexation
GASTROINTESTINAL TRANSIT	
	gastric emptying
	gut motility
	posture
	presence and type of food
	enterohepatic cycling
	disease
FACTORS AT ABSORPTION SITE	
	surface area
	barrier permeability and integrity
	membrane transport mechanism
	regional blood flow
	pH
	transmucosal metabolism
GENERAL FACTORS	
	age
	species
	sex
	body weight
	disease
	pregnancy
	diet
	pharmacogenetic modifications
	intestinal micro-organisms

Given certain limiting conditions, diffusion can be expressed by a modification of Fick's Laws as

$$\frac{dc}{dt} = - \frac{D_m A K_m}{\ell} (C_1 - C_2) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where D_m is the diffusion coefficient of the drug in the membrane, of area (A), and thickness (ℓ), and K_m is the membrane: water partition coefficient of the drug. C_1 and C_2 are the drug concentrations in the fluids on each side of the membrane. The overall rate of diffusion is also influenced by the relative volumes of the fluids, the maintenance of the integrity of the membrane and the ability of the drug to freely diffuse in either direction. Since under in vivo conditions the drug is distributed into a large blood volume and is still further distributed into the remaining intra- and inter-cellular fluids, the concentration of drug in the blood will be very low with respect to that in the gastro-intestinal lumen, i.e. $C_1 \gg C_2$. Consequently the driving force for diffusion is large and is essentially proportional to the lumenal drug concentration. The removal of diffused drug molecules from the serosal side of the membrane via the blood (i.e. drug concentration C_2) has been demonstrated to be affected by the rate of blood flow (Ochsenfahrt and Winne, 1968; Winne and Remischovsky, 1970; Crouthamel et al, 1975).

It is evident from equation (1) that the rate of diffusion is influenced by certain properties of the membrane itself. Regional differences in the rate and extent of gastrointestinal absorption can be explained in terms of the surface area of the membrane, and examination of the microstructure of the small intestine, in comparison to that of the stomach or large intestine, supports this.

The membrane: water partition coefficient of the drug is determined by its lipid solubility (hydrophobicity) and, if it is assumed that the unionised form of the drug is lipid soluble the ionisation constant of the drug and the environmental pH are determinants of that partitioning behaviour.

1.1 THE INFLUENCES OF DRUG DISSOLUTION ON GASTROINTESTINAL ABSORPTION

For drugs in solution absorption is the process limiting the onset of therapeutic activity. However, many drugs given orally are solids and have to dissolve prior to their absorption taking place. Until the 1950's and the advent of sustained-release dosage forms, disintegration testing was considered to be an adequate assessment of drug release from solid dosage forms. The subsequent development of dissolution testing methods led to the realisation that disintegration was not able to discriminate between similar, albeit subtly different, formulations since any correlation between absorption and disintegration times was, at best, tenuous. In 1957 Nelson reported that differences in the intrinsic dissolution rates of theophylline salts could provide an explanation for observed variations in their systemic availability and, since then, dissolution rate-limited absorption of many classes of drugs has been described in the literature, together with the factors influential to such behaviour. The processes of dissolution from solid dosage forms are schematically represented in Fig. 1.1.1 and have been extensively reviewed elsewhere (Wagner, 1971 ; Swarbrick, 1971; Wurster and Taylor, 1965).

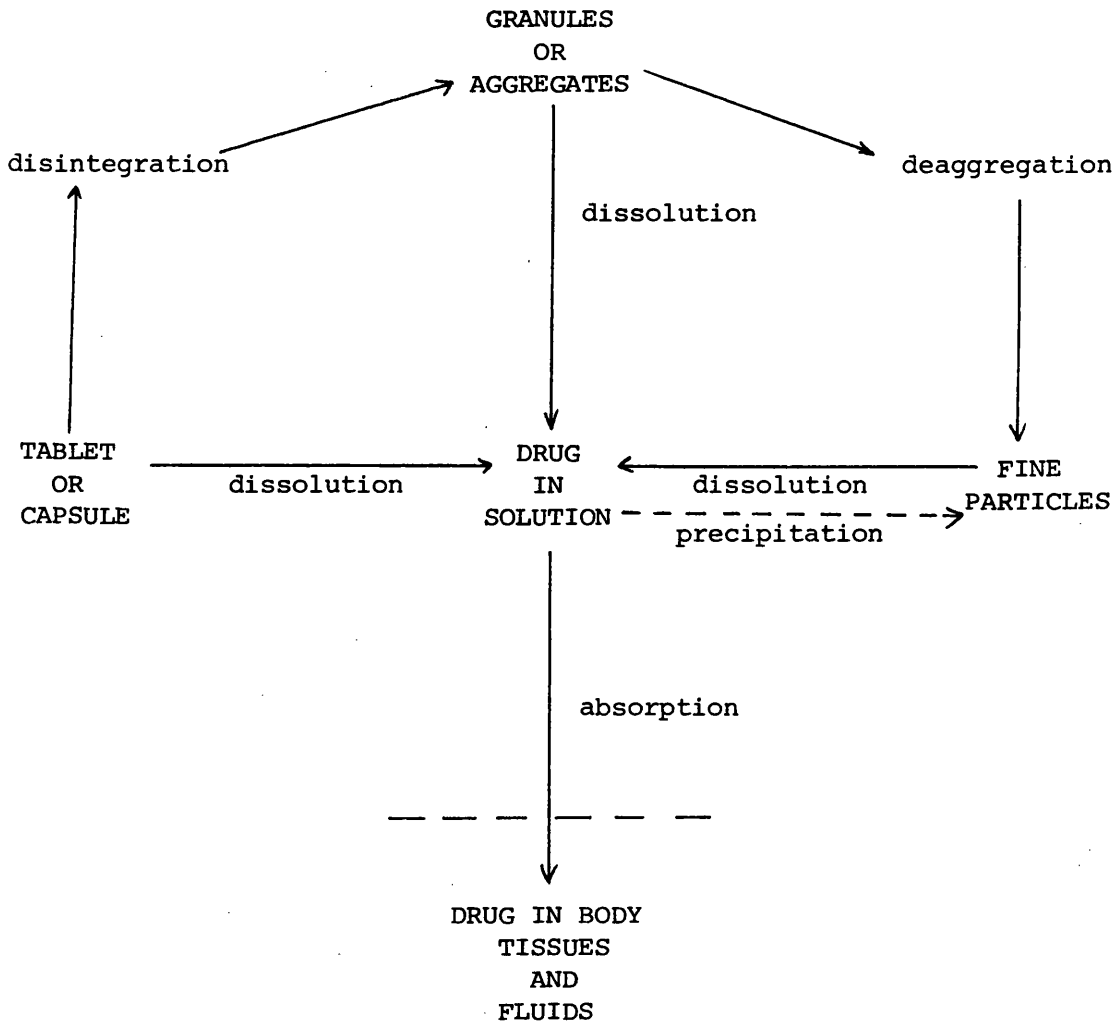


Fig. 1.1.1 Schematic Representation Of The Dissolution Processes Encountered With Various Drug Delivery Systems (from Wagner, 1971).

The relationship between the parameters controlling the dissolution process are described by progressive adaptations of the equation originally derived by Noyes and Whitney in 1897 for dissolution of solids in their own solutions:-

$$\frac{dc}{dt} = k\ (C_S - C) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$

Where C_S is the saturation solubility of the solute in the solvent, C is the concentration at time t, and k is a proportionality constant that is related to the model chosen to describe the possible mechanism of dissolution. Higuchi (1967) reviewed three mechanisms for dissolution which are depicted in Fig. 1.1.2. In the diffusion layer model (Fig. 1.1.2,A) the dissolution rate is controlled by the diffusion of dissolved material from the liquid-solid interface through the diffusion layer to the bulk solution and is, therefore, influenced by the thickness (h) of that layer. The interfacial barrier model (Fig. 1.1.2,B) describes dissolution where the non-instantaneous nature of the reaction at the solid-liquid interface is rate-limiting to the diffusion of dissolved substance into the bulk solution. Danckwerts' model (Fig. 1.1.2,C) assumes that solute transport away from the solid surface is achieved by means of macroscopic packets of solvent in contact with the surface that absorbs solute by normal diffusion and are then replaced by fresh packets of solvent.

Under well-defined experimental conditions the influences of surface area (S), diffusion coefficient (D) of the solute through the saturated diffusion layer of thickness (h) that

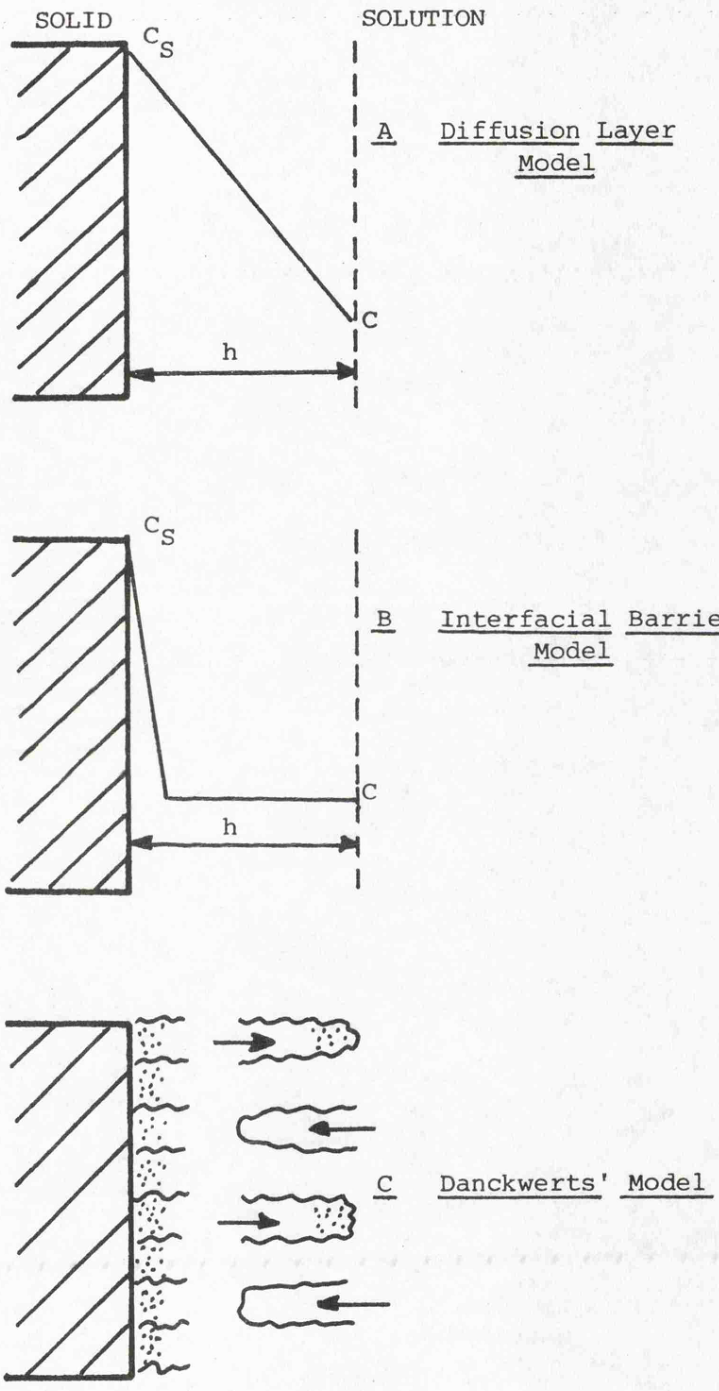


Fig. 1.1.2 Possible Models Describing The Mechanisms Of Dissolution (From Higuchi, 1967).

For explanation of the symbols refer to text.

surrounds the dissolving particle, the viscosity of the solvent (η), and the volume of the solution (V) became identified and quantifiable. These factors are incorporated into equation (2) to give:-

$$\frac{dc}{dt} = \frac{k^1 D S}{\eta V h} (C_s - C) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Although D, η, V and h are significant variables <u>in vitro</u>, under the conditions prevailing in the gut by far the greatest influence is exerted by changes in the saturation solubility and the surface area. (For discussion of the latter see 1.4.1).

1.1.1 INFLUENCE OF SOLUBILITY ON DISSOLUTION RATE

The total solubility of an ionisable acidic compound is given by equation (4)

$$C_s = [HA] + [A^-] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where the total solubility (C_s) is the sum of the intrinsic solubility of the unionised acid ($[HA]$), and the concentration of the ionised moiety ($[A^-]$). If $[HA] = C_o$ and $[A^-]$ is expressed in terms of the Henderson-Hasselbalch equation (8) then from equation (5) we get:-

$$C_s = C_o + \frac{K_a C_o}{[H^+]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

It can be seen that the solubility of an acidic compound will therefore increase with pH. The converse can similarly be shown to be true for compounds basic in nature. By substitution of equation (5) into equation (2) we get:-

$$\frac{dc}{dt} = k\left[C_o + \frac{K_a C_o}{[H^+]}\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

Equation (6) only applies under sink conditions, where C is negligible with respect to C_s (i.e. $C \ll 0.2\ C_s$), and it is evident that the dissolution rate constant (k) will increase with pH. An analogous examination similarly indicates that a decrease in pH will facilitate the dissolution rate of basic drugs.

Equations (2), (3) and (6) show that an increase in the solubility (C_s) of a drug will cause a corresponding increase in the dissolution rate.

1.2 THE INFLUENCE OF DRUG CRYSTAL HABIT AND MORPHOLOGY ON GASTROINTESTINAL ABSORPTION

The habit of a crystal is its overt appearance and is a product of environmental change on the growing crystal that results in alterations of the external shape without inducing internal, polymorphic changes. The different habits are caused by interference with the uniform approach of crystallising molecules to the various faces of the growing crystal. Irregularly-shaped crystals are described as anhedral or allotriomorphic and can be produced under adverse or changing environmental conditions associated with the degree of supersaturation, the type and temperature of the solvent and the presence of cosolvents or cosolutes. These habits are characterised and classified according to the interfacial angles which are commonly measured by a reflecting goniometer. Changes in crystal habit have been

attributed to observed alterations in syringeability, tabletting behaviour (Shell, 1963) and dissolution (Piccolo and Tawashi, 1971).

The internal structure of solid materials can be described according to whether or not a definite repeating crystal pattern is present. Any element or molecule able to crystallise as more than one distinct crystalline species is said to exhibit polymorphism; e.g. carbon can crystallise as cubic diamond or hexagonal graphite. The polymorphic forms of a given compound are, in general, as different in structure and properties as the crystals of two totally unrelated compounds and can, as such, be distinguished not only by their crystalline structure but also by their solubility, melting point, density, and optical or electrical properties. Since the production of polymorphs can result from the same environmental changes known to modify the crystal habit, the stability, solubility and absorption behaviour could be controlled by refinement of the conditions of crystallisation designed to encourage the production of specific drug polymorphs. Polymorphism has been demonstrated in a large number of groups of pharmaceutical compounds including the sulphonamides, barbiturates and steroids (Kuhnert-Brandstatter, 1965). More recent reports have shown a relationship between the polymorphic behaviour and in vivo absorption of chlortetracycline hydrochloride (Miyazaki et al, 1974), tetracycline (Miyazaki et al, 1975) and sulphamethoxydiazine (Khalafallah et al, 1974). Polymorphic transition of digoxin, induced by ball-milling, has been shown to improve the solubility of this cardiac glycoside (Florence and Salole, 1976).

It should however be noted that enhancement of drug absorption is not always a consequence of the use of polymorphic analogues that show enhanced dissolution or solubility, since rapid conversion of a metastable polymorph to a more thermodynamically stable and less soluble crystal can often occur. Furthermore, two polymorphic forms of a drug maybe identifiable in terms of structure but show no differences on the basis of their dissolution or absorption behaviour (Gunning et al, 1976).

As well as being able to exist in more than one crystal form some solids can exist without any definite internal structure, and are described as amorphous. Amorphous solids can be considered as supercooled liquids in which the molecules are arranged in a random manner somewhat as in the liquid state. The intrinsic solubility of an amorphous form of a compound is usually greater than that of the corresponding crystalline form. However, because the thermodynamic instability of amorphous drugs in aqueous suspensions promotes their reversion to a less soluble, albeit more stable, form they have found restricted therapeutic use. The slow reversion of amorphous novobiocin in aqueous suspension to a more stable, less soluble, crystalline form at first precluded its therapeutic potential. This was overcome when, in an examination of several agents likely to reduce this reversion, methyl-cellulose and polyvinylpyrrolidone were shown to be able to completely suppress the transition (Mullins and Macek, 1960). In the same paper it was also demonstrated that amorphous novobiocin exhibited an absorption in man greater than that for an equivalent dose of the crystalline form. Similarly, the

oral absorption of the amorphous form of chloramphenicol-palmitate (Aguiar et al, 1967) has been shown to be greater than that of the crystalline counterpart.

Crystallisation of substances from different solvents can result in changes in their crystalline structure due to incorporation of solvent molecules, leading to the production of solvates; these are termed hydrates when water is the solvent. The solubility and rates of dissolution of solvates are usually lower than that for the non-solvated form. Using mean human serum levels Poole et al (1968) demonstrated that the oral absorption, from aqueous suspension, of the anhydrous form of ampicillin exhibited a more rapid onset, higher intensity of effect and longer duration of action than the corresponding trihydrate. However, these two forms of ampicillin showed bioequivalence (equal rate and extent of absorption) when given orally as tablets (Hill et al, 1975). The differences in absorption of the suspended and tablet dosage forms for anhydrous and trihydrate ampicillin may be of little consequence since a large variability in the extent of ampicillin absorption, ranging from 30-70%, has been reported for any single dosage form (Loo et al, 1974; Macleod et al, 1974; Jusko and Lewis, 1973).

Identification and standardisation of polymorphic, amorphous, anhydrous and solvated forms of compounds intended for therapeutic evaluation are essential requirements for reducing batch-to-batch and manufacturer-to-manufacturer variations in drug performance. If any pharmaceutical or formulatory

manoeuvres involve dissolution and subsequent precipitation or crystallisation from any solvent then changes in the crystalline nature of the product have to be investigated.

Whereas the overt habit of a microcrystal can be examined by light microscopy, the internal crystalline nature can be distinguished by the ability of crystals to diffract X-rays. X-ray diffraction can not only differentiate between crystalline and amorphous solids, but, since polymorphic and solvated forms also have differing crystal structure, they too can be separately identified by their diffraction patterns.

1.3 CHEMICAL INFLUENCES ON GASTROINTESTINAL DRUG ABSORPTION

1.3.1 PARTITION COEFFICIENT

If it is assumed that drug absorption proceeds across a lipid barrier by passive diffusion down a concentration gradient then the rate of transport across the membrane barrier is influenced by the degree of partitioning of the drug molecules into the membrane and subsequent removal via the circulatory and lymphatic systems. Drug partitioning into lipid from the aqueous intralumenal environment can be modelled by the ability of the drug to distribute itself between two immiscible solvents, and can be expressed as the partition coefficient (K) thus:-

$$K = \frac{C_1}{C_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

where C_1 and C_2 are the equilibrium concentrations of the drug in solvents 1 and 2 respectively. The relationship expressed in equation (7) only applies to dilute solutions where

activity coefficients become negligible and where the same molecular species is found in both solvents. The relative concentrations in both solvent phases will obviously be subject to change by variations in temperature, the presence of cosolutes or cosolvents and the concentration of the molecular species common to both phases. Under in vivo conditions the partitioning of a drug in solution in the gastrointestinal fluids will be affected by its degree of ionisation, tissue and plasma binding, and drug-gastrointestinal tract interactions. However, low or negligible drug partitioning into organic solvents can be indicative of permeability-limited absorption.

The chloroform/water partition coefficient for a series of barbiturates has been correlated with the extent of their colonic absorption in the rat (Schanker, 1959). Since the barbiturates were essentially present as their unionised monomer, changes in ionisation did not influence their partitioning behaviour and absorption could therefore be related to lipid solubility. Similar rank order correlations have been established between lipid solubility and intestinal absorption for a number of basic and acidic drugs (Hogben et al, 1958). These observations form the basis of the pH-partition hypothesis for drug absorption (Hogben et al, 1957; 1958; Schanker et al, 1957; 1958; Shore et al, 1957).

Consideration of the in vitro use of ether, octanol, chloroform or heptane led Hogben (1971) to suggest that it may be more appropriate to use an organic phase that simulates the lipid nature of the cell membrane. However, Hansch and Dunn (1972)

in a review of the large amount of published data relating to in vitro/in vivo partition correlations concluded that a wide variety of in vivo partitioning phenomena could be adequately described by partition coefficients determined in octanol/water systems.

1.3.2 IONISATION CONSTANT

Ionisation of acidic or basic drugs in aqueous solution will occur to a degree dictated by the ionisation constant (pK_a) of the drug and the environmental pH. The relationship between these two parameters is expressed in the Henderson-Hasselbalch equation, which states that, for an acidic drug:-

$$pH = pK_a + \log \frac{\{\text{ionised}\}}{\{\text{unionised}\}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

and for a basic drug:-

$$pH = pK_a + \log \frac{\{\text{unionised}\}}{\{\text{ionised}\}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

As lipid solubility is primarily a characteristic of the unionised moiety, the importance of environmental pH and drug pK_a in determining the extent of lipid partitioning of the drug, and ultimately its absorption, is clear. It follows therefore that weakly acidic drugs should be preferentially absorbed from the acidic gastric regions, whereas absorption of weakly basic drugs should benefit from the higher pH of the intestinal environment. However, other factors such as differences in the absorptive surface area of the various regions of the gut and the solubility of the drug in the unionised form must be taken into account. The results of perfused in situ rat intestine

experiments (Schanker et al, 1958) suggest two pertinent conclusions. Firstly, that the mucosal barrier is preferentially permeable to the unionised drug species, and secondly, that the absorptive processes occurring in the stomach and the small intestine are essentially the same. Although, while absorption of bases does take place preferentially from the small intestine, where ionisation of the drug is retarded, absorption of acids from the intestine is often equivalent to or greater than their gastric absorption even though the drug is primarily present as the ionised species. That is not to say that the ionised species is absorbed, only that absorption of the small amount of unionised species present is rapid over the large membrane surface area, leading to re-establishment of the equilibrium between ionised and unionised forms and hence further absorption of the newly-created unionised species.

Transmembrane diffusion, however, has been demonstrated in the gastrointestinal tract for fully-ionised drug molecules e.g. tetracycline (Pindell et al, 1959). Furthermore, such ionic transport has been associated with drug complexation with endogenous membrane components and excipient counter ions for a number of fully ionised drugs and has been shown to both enhance (Irwin et al, 1969; Levine and Pelikan, 1961; Tomlinson and Davis, 1976) and retard (Levine et al, 1955; Schanker, 1960; Plakogiannis et al, 1970) their absorption. It should however be pointed out that, although the use of excipient counter ions increased the loss of drug from the lumenal side of the absorptive membrane (the situation reported by the work of Tomlinson and Davis), this is in no way a

measure of absorption since the appearance of drug in the blood was not monitored and could therefore not be inferred. In this case the ion-pair complex could also be strongly bound within the membrane and unable to diffuse into the serosal fluids.

1.4 FORMULATORY INFLUENCES ON GASTROINTESTINAL DRUG ABSORPTION

Barr (1973) intimated that drug products showing poor bioavailability* generally have a greater variation in their observed therapeutic response. This variation is of both inter- and intra-subject origin and can be exacerbated by alterations in the physical and chemical characteristics resulting from separate batches or manufacturers of the drug. The specific example cited by Barr is that of the difficulties associated with the day-to-day management of convulsive disorders by the oral use of the poorly-soluble drug, phenytoin. The suggestion is that drugs exhibiting dissolution rate-limited absorption are more likely to show an erratic response than drugs that are rapidly dissolved and completely absorbed.

Improvement in the dissolution and absorption behaviour of poorly-soluble drugs can be effected by several means. These are primarily directed towards increasing the surface area or improving the solubility of the drug in the diffusion layer.

Footnote* Bioavailability is used here, and subsequently, to indicate measurement of both the rate and extent at which an administered drug reaches the general circulation from its site of absorption.

1.4.1 REDUCTION IN PARTICLE SIZE

A reduction in the particle size of a given weight of drug will increase the specific surface area and usually results in an increase in the dissolution rate. When absorption is dissolution rate-limited it will often be enhanced by the use of the drug of much smaller particle size. This has been demonstrated for many drugs including aspirin (Martin, 1971; Parrott, 1975), digoxin (Jounda and Sothmann, 1973), sulphisoxazole (Fincher et al, 1965), griseofulvin (Kabasakalian et al, 1970; Chiou and Riegelman, 1971), phenytoin (Neuvonen et al, 1977) and three sulphonamides (Kaneniwa and Watari, 1974). Conversely, a therapeutically more useful absorption could be the result of an increase in the size of the administered material. This is exemplified by the alleviation of the gastric intolerance of finely-divided nitrofurantoin by the oral administration of the drug in macrocrystalline form. The urinary excretion of the drug from both particle size preparations did not reflect any significant differences in the extent of their absorption (Conklin and Hailey, 1969).

1.4.2 INCREASE IN DRUG SOLUBILITY

Previous discussion (see 1.2.2) has implicated polymorphism, amorphism and solvation of a drug with changes in dissolution rate and absorption by alteration of the saturation solubility. An increase in solubility can also be obtained by solubilisation of the drug into micelles, although, this may not be associated with increased absorption since the drug within the micelle is unavailable to the absorptive membranes. The use and mechanisms of surface active agents that alter gastro-intestinal absorption

have been adequately reviewed by Gibaldi and Feldman (1970).

Increased dissolution rate associated with an increase in the saturation solubility of the drug in the microenvironment of the dissolving particle can be induced by changes in the ionic composition of the diffusion layer. This is illustrated by the formulation of aspirin into solid dosage forms containing various buffer salts. These and other aspirin formulations are reviewed by Martin (1971). Soluble salts of acidic and basic compounds generally exhibit a greater dissolution rate than their respective conjugate acid or base at an equal pH. The mechanism of this effect is due to an increase in the buffering capacity of the diffusion layer resulting from cation or anion release from the ionisation of dissolved acid or base respectively. Although the use of the salt form of a drug usually increases its dissolution, studies with aluminium aspirin (Levy and Procknal , 1962), Warfarin sodium (O'Reilly et al, 1966) and benzphetamine pamoate (Higuchi and Hamlin, 1963) in tablet form showed that the salt form had a slower dissolution and subsequent absorption when compared to the corresponding free acid or base. These decreases in absorption and dissolution appeared to result from the precipitation of a less soluble film on the surface of the dissolving tablets preventing their deaggregation and reducing the effective surface area of the drug to the dissolving fluids. However, the successful therapeutic use of various salts is illustrated by absorption studies on phenoxymethylpenicillin (Colquhoun et al, 1957), barbiturates (Anderson, 1964; Nelson, 1958), sulphonamides (Nelson, 1958), novobiocin (Furez, 1958) and aminosalicylic acid (Pentikainen et al, 1974).

The therapeutic potential and drawbacks associated with the use of drugs in their salt form has recently been reviewed (Berge et al, 1977) and the oral use of soluble salts of weakly acidic drugs has been questioned on the grounds that, under the acid conditions of the stomach, precipitation of the free acid would occur to an extent dependant on the ionisation constant and concentration of the drug and the volume and pH of the in vivo environment. This is illustrated by the unexpectedly erratic urine levels of reserpine, following its oral administration to rats as a solution of reserpine acetate (Stupak et al, 1974). The observed variation in urinary excretion was attributed to incomplete absorption resulting from its gastrointestinal precipitation.

The possible role of endogenous materials has been suggested in the prevention or retardation of in vivo precipitation and in the maintenance of a supersaturated solution of the unionised form of aspirin (Leonards, 1962; Martin, 1971) and phenobarbitone (Finholt and Solvang, 1968). The occurrence of gastrointestinal precipitation, and its prevention, have received increasing attention particularly with reference to the prospective involvement of both endogenous (mucin, bile etc) and exogenous (polymers, surface active agents etc), substances (Finholt and Solvang, 1968; Solvang and Finholt, 1970; Stevens, 1973; Stevens and Padfield, 1976).

1.4.3 COPRECIPITATION

Formulatory methods aimed at increasing the dissolution of poorly-soluble compounds have progressively improved over the

last 15 years. The applicability of eutectic mixtures to pharmaceutical systems was postulated by Goldberg et al (1965) and increases in the rate of dissolution were demonstrated for eutectic mixtures of paracetamol and chloramphenicol with urea (Goldberg et al, 1966a; 1966b) and griseofulvin with succinic acid (Goldberg et al, 1966c).

The incorporation of poorly-soluble drugs on to a pharmacologically inert 'carrier' has been attempted in order to provide a greater surface area of the drug for dissolution. This technique was adopted by Monkhouse and Lach (1972a; 1972b) who reported an increased dissolution of hydrochlorothiazide, indomethacin, griseofulvin, chloramphenicol, reserpine, aspirin and sulphaethidole from their molecular dispersion on fumed silica dioxide.

Similarly the use of bile acids as 'carriers' was reported by Stoll et al (1969) for a reserpine:cholanic acid system, where the method of preparation was to dissolve both components in a common solvent and to subsequently remove the solvent by evaporation. This, and similar techniques, have assumed the title of 'coprecipitation' and has been successfully applied to a number of drugs showing dissolution rate-limited absorption. The reserpine:cholanic acid study was based on previous observations by the same group of workers (Malone et al, 1966; Decato et al, 1969) that reserpine dissolution and absorption was enhanced after its coprecipitation with a series of unconjugated bile acids (cholanic, cholic, deoxycholic, lithocholic and trihydroxycholane). These studies suggested

that the enhanced dissolution from bile acid coprecipitates was mediated through an increased surface area of the drug. This concept is supported by Stoll et al (1973) who prepared a nitrofurantoin:deoxycholic acid coprecipitate and concluded from dissolution and human urinary excretion data that the improved nitrofurantoin availability from the coprecipitate (over that of the pure drug or a drug:bile acid physical mixture) could be attributed to the dispersion of fine drug particles over a large surface area of bile acid. They further suggested that the bile acid formed a matrix around the finely-divided drug and, upon dissolution in alkaline media, the bile acid dissolved rapidly leaving an even smaller nitrofurantoin particle to dissolve in what was then a solution of bile salt.

The presence of bile acids or salts in the gastrointestinal or in vitro environment can improve drug dissolution by mechanisms other than those resulting from coprecipitation. As previously suggested (See 1.1.1), drug dissolution and the increased wetting of powders can also be induced by the concomitant use of physiological surface active agents. Since duodeno-gastric reflux is a common occurrence (Kallner, 1975), especially under fasting conditions (Rhodes et al, 1969), it can be expected that bile will be present in the stomach and will influence the dissolution and wetting of drug preparations (Finholt and Solvang, 1968). Although drug solubilisation in bile salt solutions has been shown to enhance the absorption of sulphadiazine (Nightingale et al, 1971), β-carotene, retinol (El-gorab et al, 1975), griseofulvin, glutethamide and hexoestrol (Bates et al, 1966) this need not always be so. Decreased drug

absorption resulting from complex formation with bile salts has been reported for imipramine, quinine (Kimura et al, 1972), nystatin, neomycin, kanamycin, polymyxin and vancomycin (Mahfouz, 1944; Faloon et al, 1966; Schneierson and Amsterdam, 1958).

Bile acid or bile salt interactions with the drug are not the only methods by which these surface active agents can influence absorption. Studies on the membrane effects of bile salts have shown disruption of the membrane of the rat intestinal mucosae (Feldman et al, 1973) and goldfish gills (Marriot and Kellaway, 1976) and an increased wetting of frog skin (Whitworth and Jun, 1973). Similarly the co-administration of conjugated bile acids (taurodeoxycholic acid and glycocholic) has been shown to potentiate the production of aspirin-induced lesioning of the rat gastric mucosa (Semple and Russell, 1975). Such effects were not, however, observed for the deconjugated bile acids (cholic, chenodeoxycholic and deoxycholic).

The method of drug coprecipitation with a water-soluble polymer has also been successfully employed to aid the dissolution of poorly-soluble compounds. In the mid-1960's colloidal dispersions of β -carotene in polyvinylpyrrolidone (PVP) were prepared by coprecipitation and found to increase dissolution of the vitamin (Tachibana and Nakamura, 1965). Other drugs showing improved dissolution following coprecipitation with PVP include griseofulvin (Mayersohn and Gibaldi, 1966), sulphathiazole (Simonelli et al, 1969; 1976), reserpine, digitoxin (Stupak and Bates ; 1973; Bates, 1969; Stupak et al, 1974),

acronycine (Svoboda et al, 1971), nystatin (Dexter, 1975), hydroflumethiazide and hydrochlorothiazide (Corrigan and Timoney, 1975; 1976). In all cases the coprecipitated systems were reported to be superior to the drug alone, or to an equivalent physical mixture of the components, when compared by dissolution and/or *in vivo* absorption studies. Particularly good correlations were obtained between the *in vitro* and *in vivo* behaviour of the reserpine: PVP systems (Stupak and Bates, 1974; Stupak et al, 1974). In all cases where the internal crystal structure of the drug:PVP coprecipitates was examined by X-ray diffraction techniques no evidence was found for crystallinity and the coprecipitates were considered to be amorphous. The mechanisms involved in the production of amorphous drug:PVP coprecipitates can be related to the ability of PVP to retard, and even prevent, the growth of drug crystals (Mullins and Macek, 1960; Simonelli et al, 1976; Corrigan and Timoney, 1974). However, by far the most definitive examination of drug release from PVP coprecipitates has been achieved for sulphathiazole by Simonelli et al (1969; 1976). These workers found a rank order relationship between the rate of dissolution and the molecular weight of PVP used and, by following the release of both drug and polymer from discs of constant surface area and from X-ray diffraction, solubility and cell diffusion studies, they proposed a model to describe the release of sulphathiazole and PVP from their coprecipitates. The model was based on the differential release of drug and polymer from coprecipitates of varying drug:polymer ratios and adequately described the observed dissolution behaviour throughout the range. The overriding conclusions were that the enhanced

solubility and dissolution resulting from coprecipitation with PVP is primarily a property of the amorphous nature of the final product.

1.5 PURITY AND STABILITY

Errors in dosage and differences in *in vitro* and *in vivo* behaviour of many formulated products can often result from the presence of impurities. These can be a consequence of the use of impure components during the manufacturing processes or degradation and contamination of the final product during storage, transport etc. In this context, Bundgaard (1974a) showed that acetylsalicylic anhydride impurities in discs of aspirin decreased the rate of dissolution of the aspirin to an extent related to the amount of impurity present. The same author (Bundgaard, 1974b) has also suggested that the immunogenic action of aspirin preparations could be attributed to the aminolytic attachment of glycine to trace acetylsalicylsalicylic acid impurities. Purity determinations are therefore essential in product assurance and the control of untoward therapeutic, toxicological and pharmacological actions. This is especially true if the pharmaceutical and formulatory manoeuvres employed in preparation of suitable dosage forms are capable of promoting the degradation of the active principle.

An understanding of the susceptibility of drugs to degrade is of use in the design and interpretation of pharmaceutical and absorption studies. The correlation of *in vitro* with *in vivo* degradation for a series of penicillins (Schwartz and Buckwalter, 1962) and more recently for digoxin (Gault, *et al* 1977) illustrate this clearly.

The most frequently occurring mechanisms of drug degradation are those of hydrolysis and oxidation, the latter being capable of photochemical initiation. Of greatest significance is hydrolysis, especially when the drug is present in an aqueous vehicle. The main factors that determine the rate of hydrolytic degradation are the concentrations of H^+ and OH^- ions and temperature. Both the hydroxyl and hydronium ions (H^+ exists predominantly as the hydrated H_3O^+ ion) are catalytic in most instances resulting in a stability/pH profile showing a small pH range of maximum stability. The overall rate of hydrolysis can be written as:-

$$-\frac{dc}{dt} = k_o\left[H_2O\right] + k_1\left[H_3O^+\right] + k_2\left[OH^-\right] \quad \ldots\ldots\ldots\ldots (10)$$

where k_o, k_1 and k_2 are the specific first order rate constants for the water, hydronium- and hydroxyl-ion-catalysed reactions respectively. As k_o is generally very small, under highly acidic conditions where $\left[OH^-\right]$ will be negligible equation (10) reduces to:-

$$-\frac{dc}{dt} = k_1\left[H_3O^+\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

which, ignoring activity corrections, can be written as:-

$$\log k_{obs} = \log k_1 - pH \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

where k_{obs} is the observed first order rate constant.
Similarly, in the presence of high hydroxyl ion concentrations:

$$\log k_{obs} = \log k_2 - pk_w + pH \quad \ldots\ldots\ldots\ldots\ldots\ldots (13)$$

therefore a plot of log k_{obs} against pH should show two linear regions, one with a negative slope of 1 where hydronium ion catalysis is predominant and one with a positive slope of 1 where hydroxyl ion catalysis is predominant. There will also be an intermediary region where all three terms in equation (10) make a contribution to the observed rate.

The ability of a drug to ionise will affect the overall rate constant as given by equation (14).

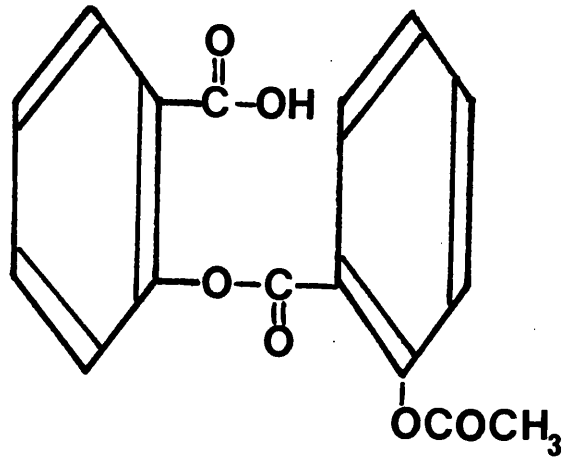
$$-\frac{dc}{dt} = [S]\left[k_o + k_1[H_3O^+] + k_2[OH^-]\right] + [SH^+]\left[k_3 + k_4[H_3O^+] + k_s[OH^-]\right] + [S^-]\left[k_6 + k_7[H_3O^+] + k_8[OH^-]\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (14)$$

where $[S]$, $[SH^+]$ and $[S^-]$ are the concentrations of the neutral, protonated and anionic forms of the parent drug. The concentrations of the different ionic species and hence their contribution to the overall rate of hydrolysis will be dependant upon the ionisation constant of the drug and the pH of the system. The change of substrate from one ionic species to another is responsible for the inflections and plateaus associated with the stability/pH profiles of aspirin (Kelly, 1970), procaine (Higuchi et al, 1950) and atropine (Zvirblis et al, 1956).

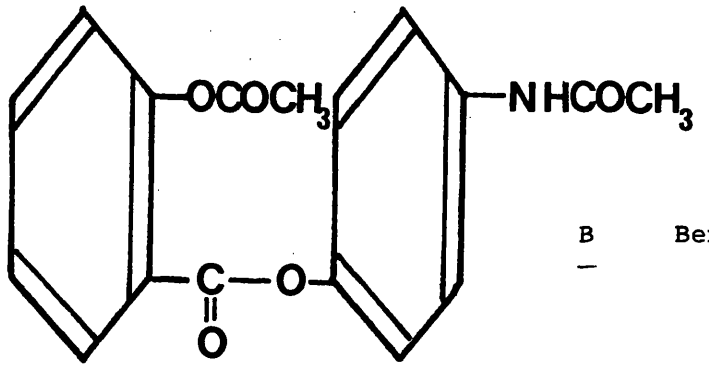
1.6 DRUG MODEL USED IN THIS STUDY

At the beginning of this century many salicylate esters and other salicylate derivatives were synthesised in an attempt to alleviate the gastrointestinal toxicity associated with the oral use of salicylic acid or its sodium salt. The most

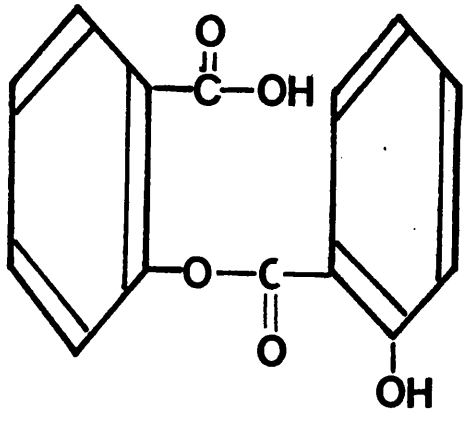
outstanding of these was the acetyl ester, aspirin, a name first used by the Bayer Company in 1899. Aspirin has appeared in many dosage forms, as different salts, in soluble, effervescent, buffered and conventional tablets, in admixtures with glycine and as aluminium derivatives (Martin, 1971). Reduction in the gastrointestinal toxicity due to salicylate therapy has also been attempted by the formulation of sustained-release and enteric-coated tablets. Changes in the rationale behind the use of salicylate homologues has warranted reconsideration of salicylate acid precursors or prodrugs. This is based on the observations including those of Bayles and Tenckhoff (1959), that the exacerbation of rheumatic symptoms after the sleeping hours could be attributed to low plasma salicylate levels produced by the use of conventional salicylate therapy. Examples of aspirin precursors are acetylsalicylsalicylic acid or Acesal (Fig. 1.6.1,A) and more recently the paracetamol ester of aspirin Benorylate (Fig. 1.6.1,B). Other reported aspirin precursors include the alkylcarbonate anhydrides, and glyceryl, hydroxyalkyl and acetomethyl esters (from Scherer, 1974). Esters of salicylic acid, other than aspirin, have been synthesised subsequent to the association of salicylic acid with its antirheumatic, analgesic, anti-inflammatory and antipyretic actions. The use of the majority of these compounds has suffered from the dominance of aspirin with respect to their commercial and therapeutic potential. They are, however, receiving increased attention particularly with a view to providing a prolonged (12 hour) action without incurring the erratic performances shown by enteric-coated or sustained-release aspirin preparations. Whitehouse (1965), in an



A — Acesal
Acetysalicylsalicylic Acid



B — Benorylate



C — Salysal, Diplosal, Disalcid
Salicylsalicylic Acid
SSA

Fig. 1.6.1 Chemical Structures Of Acesal (A), Benorylate (B) And Salicylsalicylic Acid (C).

extensive review of the biochemical and pharmacological properties of anti-inflammatory drugs, states

> ". . . it is unlikely that any simple salicylate derivative will in fact displace salicylic acid as an anti-inflammatory drug, outside of commercial considerations. The only 'super-salicylate' which might be of value as an anti-rheumatic drug will probably be a drug combining within itself the structure of salicylic acid together with some other feature(s) which also carries potential anti-rheumatic activity.".

One compound that fits the above description is benorylate (Fig. 1.6.1,B) which has been developed by the Sterling-Winthrop Company. Another compound, which is available in Scandinavia and has recently (January 1977) been marketed in this country, is the double ester of salicylic acid, salicylsalicylic acid (SSA), (Fig. 1.6.1,C). Patented by the Boehringer Company of Germany in the early 1900's, SSA has enjoyed a limited use under such names as Diplosal, Persistin, Salysal (United States), Dinuclan (France), Nobacid (Scandinavia) and recently Disalcid (United Kingdom). The observed palatability of SSA over that of sodium salicylate has been attributed to its poor solubility (Hanzlik and Presho, 1925) and limited stability studies carried out by these authors led to the suggestion that

> ". . . the acidity of the gastric juice would not decompose SSA to any appreciable extent during its time of sojourn in the stomach.".

These authors further claimed that alkaline hydrolysis was negligible and suggested that intestinal absorption would be small unless other factors such as its lipid solubility or the increased solubility of the salt (ionised) form were significant. This suggestion is based on the assumption that SSA must degrade to salicylic acid prior to its absorption. Their excretion studies with man showed that salicylate could be detected in the urine up to 72 hours following oral administration of a 4-12 g. dose and could account for about 60% of that dose. The gastrointestinal insolubility of SSA has been attributed as the cause of prolonged plasma salicylate levels (Steel et al, 1931; Bastide, 1955; Rubin, 1964; Nordqvist et al, 1965) and, the absence of occult gastro-intestinal blood loss and mucosal erosions in man (Leonards, 1969; Edmar, 1970) and the guinea-pig (Aberg and Larsson, 1970).

The indications, therefore, are that the gastrointestinal absorption of SSA is limited by its dissolution rate due to poor aqueous solubility. Moreover, since the physical and chemical characteristics of the drug are not reported, their determination would be a necessary prerequisite to an adequate description of its <u>in vitro</u> and <u>in vivo</u> behaviour. If dissolution is the rate-limiting step in SSA absorption following oral administration, then it should provide a useful model with which to investigate the possible benefits of novel pharmaceutical and formulatory manoeuvres.

CHAPTER TWO

PHYSICAL AND CHEMICAL STUDIES

2.1 MATERIALS

Salicylsalicylic acid (SSA), mol.wt. 258.08, from a single batch, was used as supplied, (A. B. Bofors Nobel-Pharma, Sweden). It is a white to almost-white crystalline powder of 'pharmaceutical grade' containing 98-101% w/w SSA, all of which should pass through a 40 mesh (400 μm) sieve (Bofors 1971).

Chemicals and reagents used throughout were of Analar grade, unless otherwise stated.

Distilled water was obtained from a double all-glass still. For preparation of pH standard solutions freshly prepared double distilled water was boiled and cooled prior to use.

Glassware. Volumetric glassware was of grade B with the exception of a 10 ml grade A microburette employed for titrimetric determinations. All glassware, when necessary, was cleaned by complete immersion in freshly prepared chromic acid, rinsed several times in tap water and five times with distilled water.

2.1.1 INSTRUMENTATION

Spectrophotometers. Ultraviolet and visible scanning spectrophotometry employed for the identification of absorbance maxima and possible cosolvent or cosolute interference in mixed systems was achieved by use of a Unicam SP1800 recording spectrophotometer in conjunction with a Unicam AR25 linear recorder. Single point fixed-wavelength absorbance determinations were obtained by use of either the SP1800 or a Unicam SP500

(series II) spectrophotometer. For ultraviolet and visible spectrophotometry 1 cm matched quartz and glass cuvettes were used respectively.

Infra-red spectrophotometry was carried out using a Unicam SP200 recording IR spectrophotometer.

Balances. An Oertling (model R20) single pan analytical balance was used for all weighings greater than 50 mg.

Temperature Control. For stability and solubility studies a waterbath fitted with a mercury contact thermostat capable of maintaining temperature $\pm$ 0.05^{o} was used (Type CT3. Laboratory Thermal Equipment, Oldham). Temperature control in other studies was achieved by the use of an insulated waterbath fitted with a thermostatically-controlled heating unit capable of maintaining temperature $\pm$ 0.1^{o} (TE4, Techne, Cambridge).

Measurement of pH. All pH measurements were made using a Radiometer type 27 pH meter fitted with a PHA925a type scale expander. The electrode used in all cases was of the combined type (Pye-Ingold E07 ref. 041). For each series of measurements within any one experimental procedure the pH meter was calibrated against two pH standards, prepared according to McGlashan (1971), that bracketed the experimental range.

2.2 X-RAY DIFFRACTION ANALYSIS

Diffraction patterns are produced whenever light passes through or is reflected by a periodic structure that has a regularly repeating feature. In order for the diffraction pattern to be prominent the repeat distance of the periodic structure should be approximately equal to the wavelength of the light used. Examination of the appropriate distances for most crystals and X-ray light shows them to be of the same order, ie 10^{-8} cm.

If two electromagnetic waves are of the same frequency and are superimposed in phase there is a summation in amplitude, termed constructive interference. If, on the other hand, the maximum and minimum amplitudes of waves are superimposed, ie they are out of phase, they cancel each other, termed destructive interference. When the two waves are partially out of phase some destructive interference occurs and there is a diminished amplitude. If the diffracted waves are changed by an integral proportion, ie by an inte ger of $m\lambda$, (where m = 0, 1, 2, 3 etc), then constructive interference results.

When X-rays of wavelength λ strike a single plane of atoms (or molecules) as in Fig. 2.2.1 and are reflected at an angle β, a summation of intensities resulting from constructive interference will ensue if the difference in the path of the adjacent rays is an integral number of wavelengths ($m\lambda$). Under these conditions it is clear that the diffracted waves will arrive at the detector in phase.

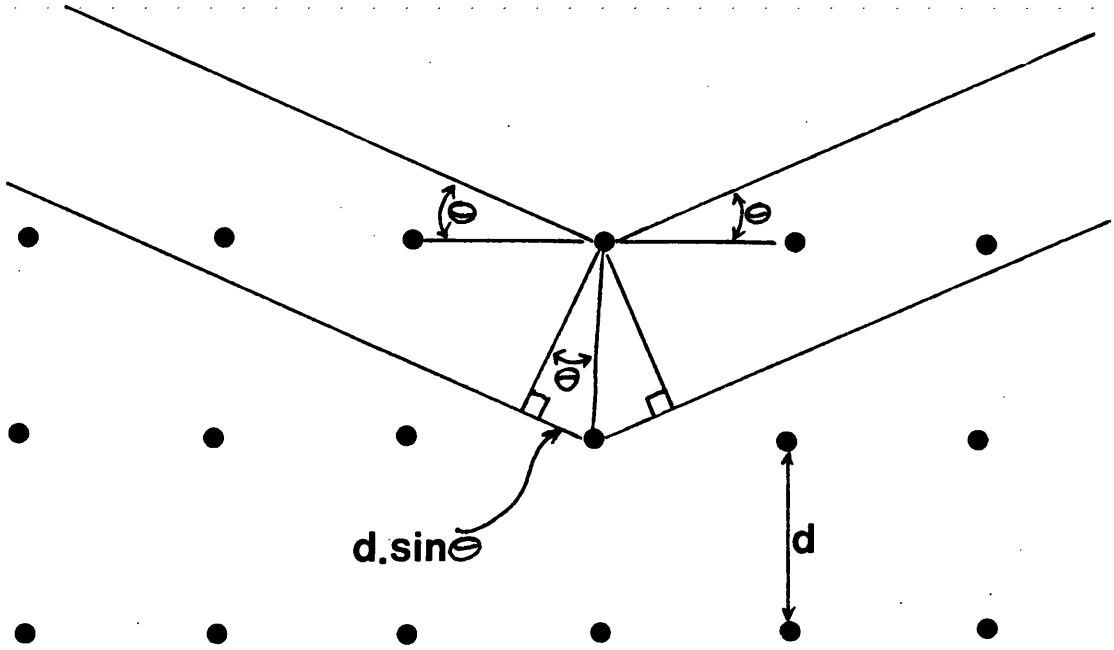


Fig. 2.2.2 Diffraction From Successive Parallel Planes Of Atoms.

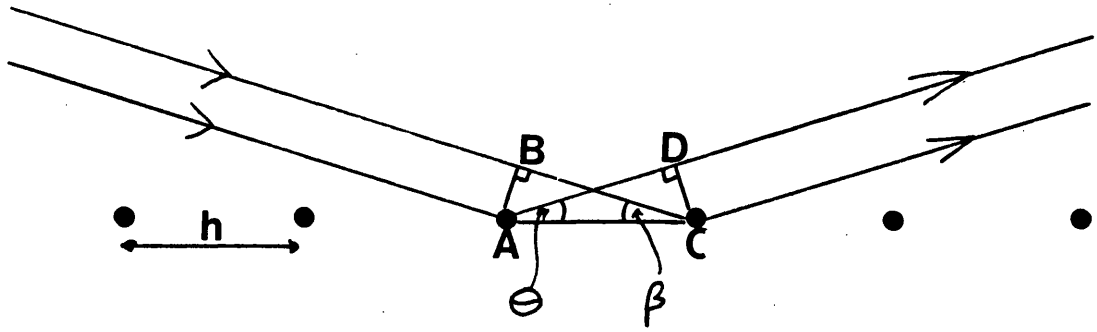


Fig. 2.2.1 Diffraction By A Row Of Equally Spaced Atoms.

From Fig. 2.2.1 it is seen that adjacent rays follow different paths and this difference AD-BC must equal $m\lambda$. Thus:-

$$AD-BC = m\lambda \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (15)$$

where $m = 0, 1, 2, 3 \ldots$ etc

Furthermore where $m = 0$ and $\beta = \theta$ it follows that:-

$$h = (\cos\beta - \cos\theta) = m\lambda \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (16)$$

therefore when the angles of the incident and diffracted beams are the same there is a maximum intensity at the detector.

Similarly for parallel planes of atoms (or molecules) the reflected (or diffracted) beams must reach the detector in phase in order for summation of intensities to occur. From Fig. 2.2.2 the path difference for the two waves is given by the Bragg equation, $2d\sin\theta$, where d is the spacing between planes. Therefore for parallel planes of atoms

$$n\lambda = 2d\sin\theta \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (17)$$

This equation has two important applications. Firstly, if the 'd' spacings of the planes of the crystal lattice are known then the wavelength of the X-rays can be calculated from the measured angle of diffraction, θ . This procedure has been used for the elements and subsequent determination of their atomic number. Secondly, if the wavelength of the incident light is known the characteristic interplanar spacings of a crystal ('d' spacings) can be computed from measurements of the diffraction angles, θ .

One basic assumption which enters the derivation of the Bragg equation is that the lattice planes are regularly spaced. If the arrangement of atoms (or molecules) in any one plane or between planes is irregular then sharp X-ray diffraction patterns are not observed.

Definitive descriptions of the internal structure of crystals is an extremely specialised science, usually centering on the study of single crystals. However for phase identification work powdered samples of material are studied by X-ray diffraction techniques. These do not require any chemical treatment of the sample and, apart from a reduction in particle size, samples can be examined as presented. Admixtures of differing crystallinity, which show no interaction, are revealed by a super-imposition of the X-ray patterns of the constituent materials. Solid solutions, polymorphic changes and amorphous substances all reveal characteristically different X-ray patterns from the parent crystal and can therefore be identified.

Apparatus. X-ray diffraction techniques have been used throughout these studies to examine for crystallinity and changes in crystallinity induced by recrystallisation in the presence of human and simulated gastric juice and PVP, and SSA coprecipitation with PVP, cholic acid and deoxycholic acid.

Two methods have been used, the choice of which was dictated by the size of the sample and the type of equipment available.

2.2.1 POWDER CAMERA STUDIES

Material for examination was finely ground in a small onyx pestle and mortar and packed into the end of a 0.5 mm 'Lindermann' glass capillary tube (Pantak Ltd, Berks) by gentle tapping. The capillary was then secured on a rotating spindle, centred in a powder film camera (Debye-Scherer, 114.8 mm diameter) loaded with X-ray sensitive photographic film (Kodirex) and the whole mounted on a Philips PW1010/80 generator capable of producing Cobalt Kα radiation (wavelength 1.79021 x 10^{-8} cm). Exposure times for all samples were between 12 and 16 hours. A diagrammatic representation of the type of powder film camera used is shown in Fig. 2.2.3. Resultant films were appropriately developed and examined for bands of diffraction. The relative intensities of the bands were subsequently obtained from densitometer scanning of the films.

2.2.2 POWDER PLATE STUDIES

Essentially the same principles apply to the powder plate diffraction techniques as for the Debye-Scherer film camera except that detection of the diffracted rays is achieved by a direct measurement of their beam intensity, either by means of the ionisation produced in a gas, or the fluorescence produced in a solid. The monochromatic light source is focussed on a centrally-positioned flat sample holder and the X-ray detector (or counter) is placed on the circumference of a circle centred on the sample. The schematic features of the X-ray diffractometer are shown in Fig. 2.2.4. The supports for the detector and the specimen holder are mechanically coupled so that rotation of the detector through 2x degrees is accompanied

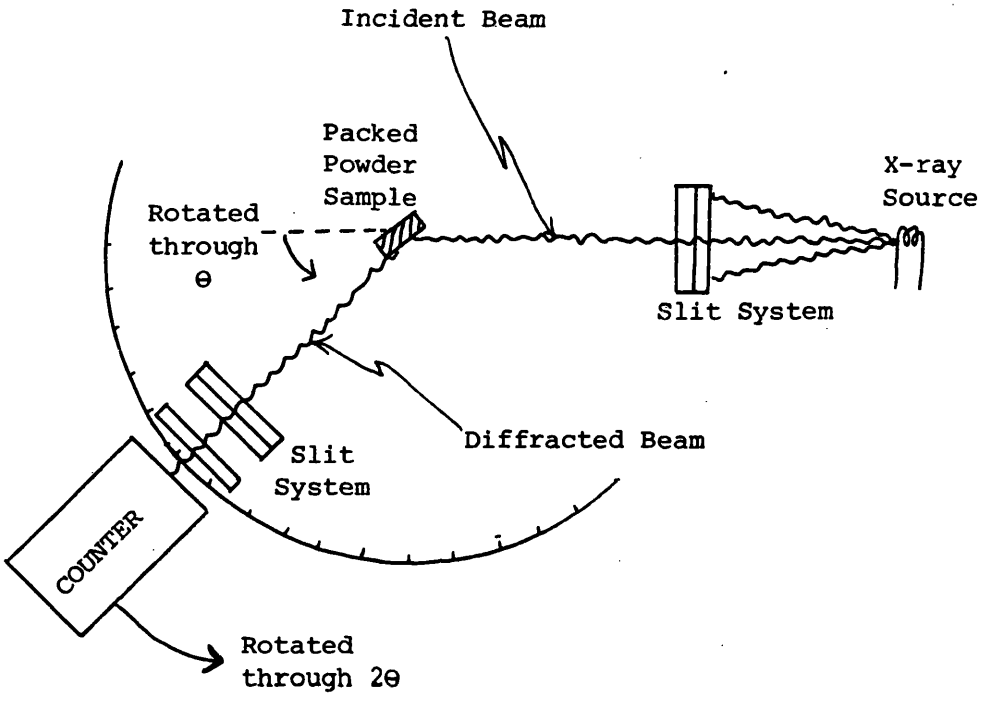


Fig. 2.2.4 Schematic Diagram Of A Powder Plate Diffractometer

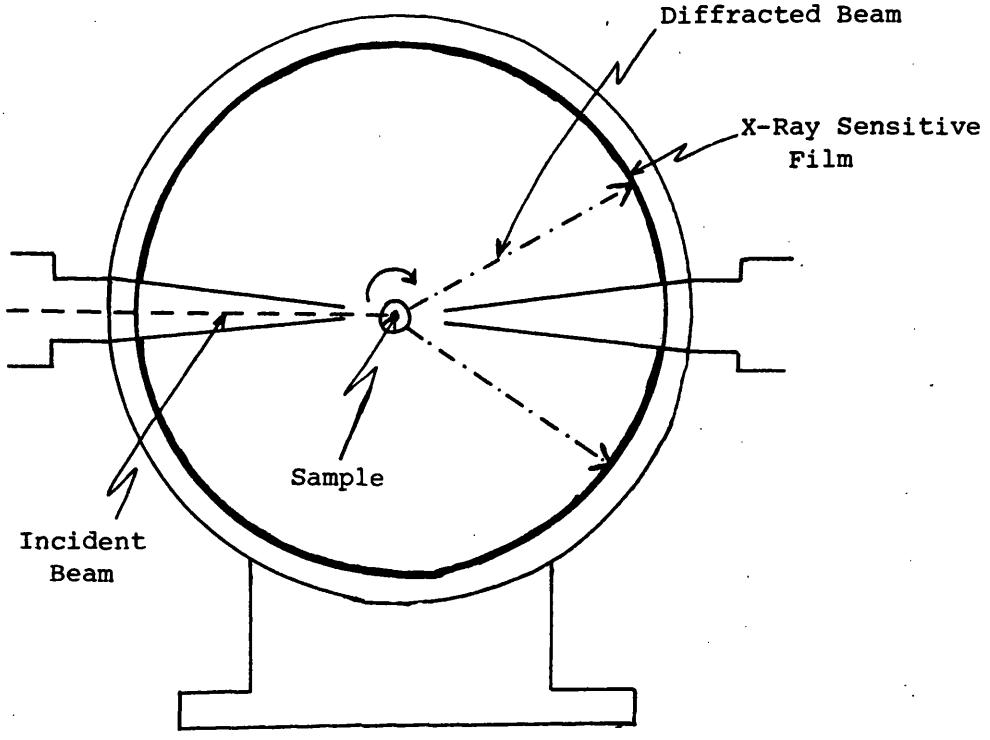


Fig. 2.2.3 Schematic Diagram Of A Debye-Scherrer Powder X-Ray Diffraction Camera.

by rotation of the specimen through x degrees, an arrangement necessary to maintain correct focussing conditions. The detector is usually linked to a flat-bed recorder synchronised to movement of the detector through increasing values of 2θ, producing a chart recording of X-ray diffraction intensities in a position proportional to 2θ. Consequently the 'd' spacings can be calculated by use of the Bragg equation (17).

Larger amounts of material were required for X-ray diffractometer analysis than for the film camera techniques and could therefore be used for X-ray diffraction analysis where quantities in excess of 2.0 g was available. 1 - 2.0 g of material was finely ground in a glass mortar and placed in a flat metal holder in such a manner as to avoid any preferred orientation of the particles. The specimen, in its holder, was then fixed into the diffractomer (Philips PW1050) and diffracted Copper Kα X-rays (wavelength 1.5418×10^{-8} cm) were detected by means of a Xenon probe counter with an appropriate pulse height discriminating facility to reduce background interference. A typical diffractometer recording is shown in Fig. 2.3.5.1.

2.3 DETERMINATION OF PURITY

2.3.1 INFRA-RED SPECTRUM

A potassium bromide/SSA disc was prepared from oven dried material and the IR spectrum obtained was qualitatively identical to that of the reference spectrum provided (Bofors, 1971). The wavelength of the peaks numbered in Fig. 2.3.1.1 are compared with those for the reference in Table 2.3.1.1.

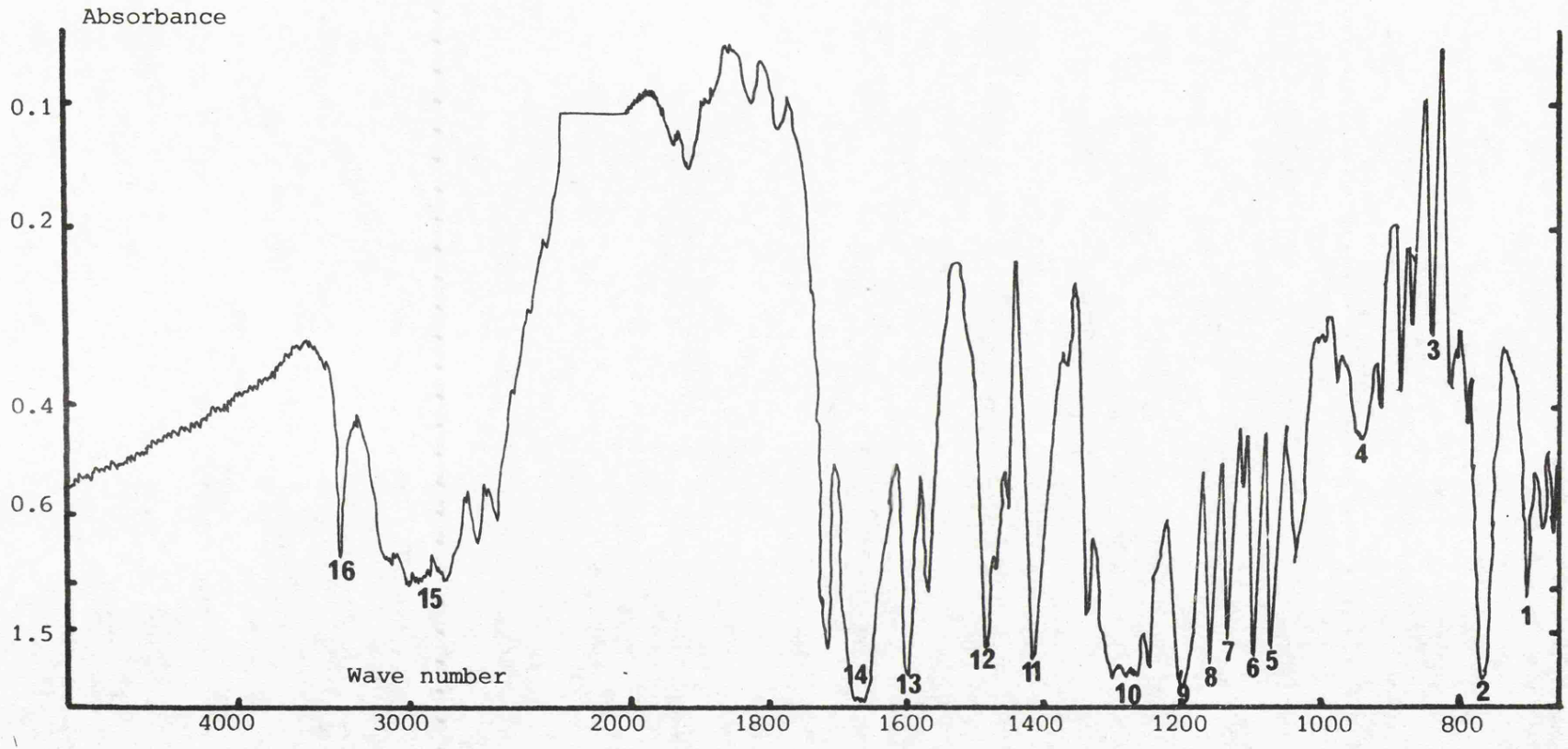


Fig. 2.3.1.1 Infra-red Spectrum For SSA Obtained Using A KBr Disc.

	Wavelength (nm) for peaks No.															
Peak No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Reference	14.4	13.2	12.1	10.7	9.3	9.2	8.8	8.6	8.4	8.0 to 7.7	7.1	6.8	6.3	6.0	3.5 to 3.2	2.9
Sample	14.4	13.0	12.0	10.7	9.3	9.2	8.8	8.6	8.4	7.9 to 7.7	7.1	6.8	6.3	6.0	3.7 to 3.2	2.9

Table 2.3.1.1 Comparison of Infra-Red Spectra Peaks of SSA For the Reference and Experimental Spectra

2.3.2 THIN LAYER CHROMATOGRAPHY

TLC identification of SSA and other impurities in samples of salicylic acid has been previously reported (Bailey, 1964; Skelly, 1966). The method used by Bailey (1964) was adopted for the qualitative identification of salicylic acid as an impurity in SSA.

A silica gel G:water (1:2) slurry was prepared and spread to a depth of 0.25 mm on 20 cm x 20 cm glass plates. The plates were air dried, activated at 105^{o} for one hour in a hot air oven and stored over silica gel in desiccator until cool. Solutions of a salicylic acid standard and SSA were prepared in ethanol to give a final concentration of 10 $mg.ml^{-1}$. Five 5 μl aliquots of each were spotted 4 cm from the base of the plates using an Agla micrometer syringe (Burroughs Wellcome) fitted with a flat needle. Each spot therefore contained 250 μg of material.

The plates were developed using petroleum ether:ethyl acetate: glacial acetic acid (85:10:5) in circular glass tanks lined with Whatman No 1 chromatography paper. The tanks were allowed to stand for two hours before use to ensure equilibriation of the solvents with their vapours and to promote even development the tanks were placed in a dark cupboard. When the solvent front was within 3 cm of the top of the plate, the plates were removed, the solvent front marked, and air dried in a fume-cupboard. The spots were visualised either by examination of the plates under long wavelength ultraviolet light (Allen Co, London, Type A409) or by spraying with a solution of 1% w/v potassium permanganate in 1% w/v sodium hydroxide. Further

differentiation between SSA and salicylic acid was achieved by spraying with ferric reagent (See 3.2.2) which produced a purple chromophore with salicylic acid only.

Result. From the TLC analysis no salicylic acid impurities in SSA were identifiable, and reproducible Rf values of 0.35 and 0.52 were obtained for SSA and salicylic acid respectively.

2.3.3 TITRIMETRIC DETERMINATION

About 200 mg SSA was accurately weighed into a 250 ml conical flask and completely dissolved in 25 ml of absolute ethanol. This was titrated against standardised 0.1N sodium hydroxide from a grade A microburette using bromothymol blue (0.1% w/v in 50% v/v ethanol) as an indicator. Before the endpoint 25 ml of water was added and the titration continued until a constant blue colour was produced. The percent purity was calculated according to the relationship.

1 ml 0.1N NaOH $\equiv$ 0.025808 g SSA

therefore

$$\% \text{ purity} = \frac{A.N.f.\ 0.025808}{w} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (18)$$

where A is the volume of NaOH of normality (N), f is the correction factor and, w is the weight of SSA taken (in grammes).

From the data tabulated overleaf (Table 2.3.3.1) the % purity was calculated to be $100.42 \pm 0.21\%$

No.	wt. SSA (g) W	Vol. 0.1N NaOH (ml) A	% Purity
1	0.2302	8.99	100.79
2	0.2268	8.84	100.59
3	0.2045	7.98	100.71
4	0.2062	8.02	100.38
5	0.2020	7.80	99.65
		$\bar{x}$	100.42
		SD	0.46
		SE	± 0.21

Table 2.3.3.1 Assessment of SSA Purity by Titration

2.3.4 MELTING POINT

An initial estimate of the melting point of SSA was obtained using the capillary tube method (Melting Point Apparatus, Gallenkamp). A more accurate determination on a hot bench (Type 7841, Shandon) using the initial estimate of 152° to give an indication of the range over which to calibrate the bench. Benzaldehyde and phenacetin with melting points of 163° and 134° respectively were used as standards and repeated determinations with SSA gave the melting point as 152-153°C. Values from the literature are given in Table 2.3.4.1 with their appropriate source.

Melting Point °C	Literature Source
135-139	Bofors (1971)
141-143	Patel et al (1972)
144-148	Kuhnert-Brandstatter (1971)
148-149	Dictionary of Organic Compounds (1965)
147	Utermark and Schike (1963)
147	Hanzlik and Presho (1925)
141-143	Pharm. Helv. (1934)

Table 2.3.4.1 Literature Values for the Melting Point of SSA.

2.3.5 CRYSTALLINITY

X-ray diffraction patterns for SSA, as supplied, and after recrystallisation from chloroform, ethanol and methanol were obtained by the powder plate method as previously described (See 2.2.2) and are shown in Fig. 2.3.5.1. The 'd' spacings and relative peak intensities, calculated from the recording Fig. 2.3.5.1,A by use of the Bragg equation (17), are given in Table 2.3.5.1 and provide an X-ray diffraction standard for future reference.

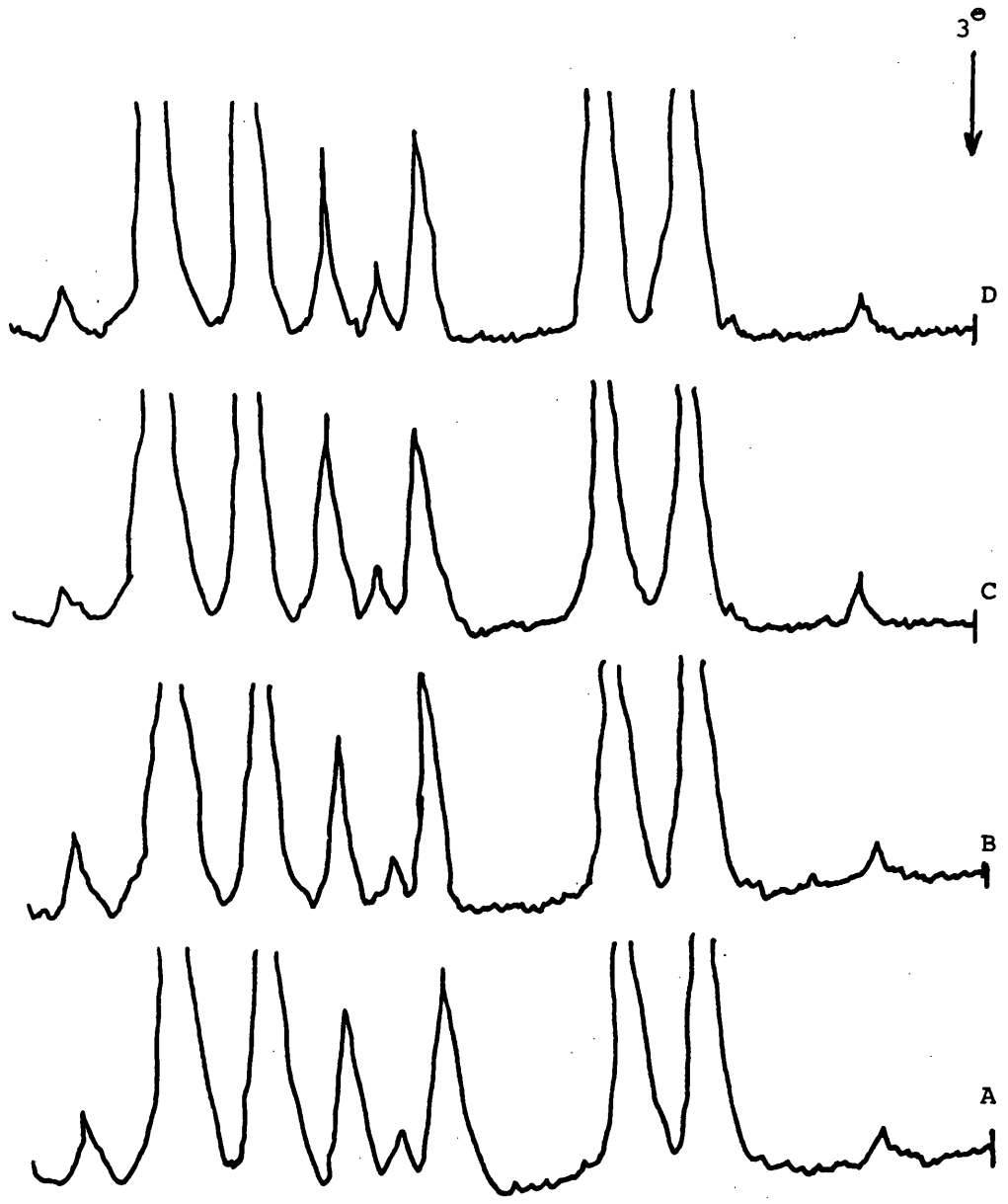


Fig. 2.3.5.1 X-Ray Diffraction Patterns For SSA As Supplied (A) And Recrystallised From Chloroform (B), Methanol (C) And Ethanol (D).

Peak No	'd' spacing (A^{O})	Peak height (mm)	Relative intensity (% max)
1	9	8	5.23
2	6.7	153	100
3	5.9	120	78.4
4	4.85	43	28.11
5	4.7	7	4.58
6	4.55	29	18.95
7	4.1	152	99.35
8	3.8	86	56.21
9	3.55	4.5	2.94

Table 2.3.5.1 'd' Spacings and Relative Peak Intensities for the X-ray Diffraction of SSA (as supplied).

It is clear that recrystallisation from the three organic solvents did not result in any changes in the crystalline nature of the drug. The similar nature of the diffraction peaks is also indicative that products of solvation with the organic solvents were not formed.

2.4 PARTICLE SIZE DISTRIBUTION AND SURFACE AREA

A large number of methods are available for measurement of particle size, especially of powdered materials, which have been reviewed by Allen (1974) and Edmundson (1967) both of whom indicate that the classification of powders into size ranges is somewhat dictated by the method of measurement. The sieve range extends from approximately 45 μm to a practical upper limit of approximately 1680 μm and powders falling within this range are termed 'coarse' (B.P. 1973). Micromesh sieves, made by electro-plating and photoengraving techniques, are available and can extend the sieve range down to 10 μm. Methods other than

sieving, based on microscopy, sedimentation, air permeability and the Coulter Counter have a useful sensitivity in the sub-sieve (45 - 1 μm) range. A third, submicron range, is receiving increased attention and analytical methodology for this submicron range includes electron microscopy, X-ray diffraction, gas adsorption and centrifugal sedimentation.

According to the standards provided for the particle size of SSA (Bofors, 1971) 100% should pass through a 40 mesh (400 μm) sieve.

2.4.1 DETERMINATION BY SIEVING

Particle size determinations using sedimentation and the Coulter Counter techniques were discounted due to problems of inadequate wetting, a lack of a suitable electrolyte or suspending system, and, unknown SSA stability in aqueous environments.

Sieving was carried out in accordance with British Standard No 1796 (B.S. 1952) using sieves of British Standard specifications (B.S. 1963). A nest of 6" sieves (Endecotts Ltd, London) was used on a mechanical sieve shaker fitted with an automatic time switch (Pascall Engineering Co, Crawley). The nest was composed of nine sieves that ranged from 40 mesh (400 μm) down to 300 mesh (53 μm). These were individually weighed empty before the sieving procedure started. 100 g of SSA was placed on to the top sieve and the nest shaken for 15 minutes. The top sieve was removed, weighed with its contents, replaced and shaken for a further five minutes. The top sieve was then again removed and weighed. If the difference between the first and second

weighings was greater than 0.2% of the total powder weight (100 g) the five minute shaking process was repeated. However if the difference was less than 0.2% the sieve was replaced and the five minute shaking and weighing cycle was repeated for the next sieve in the nest. This process was continued throughout the nest until complete. To prevent occlusion of the mesh each sieve was brushed with a brass sieve brush between each cycle. The final weight of material remaining on each sieve, ie amount oversize, was recorded. The subsieve fraction was transferred to a nest of micromesh sieves (Endecotts Ltd, London) and the shaking and weighing process repeated as before. The resultant sieve fractions were stored in clean dry glass containers in a desiccator.

The weight of material oversize for each sieve fraction is shown in Table 2.4.1 and the results are graphically represented in Fig. 2.4.1 as the cumulative % undersize vs sieve range. From this frequency ogive a median value of 78 μm is obtained. To establish whether the distribution of particles follows a normal or logarithmic pattern the cumulative % undersize as probability on the ordinate was plotted against the arithmetic mean or its logarithm on the abcissa as shown in Figs. 2.4.2 and 2.4.3. From these graphs it is evident that the particle size distribution of SSA follows a logarithmic normal pattern. This type of particulate distribution is characteristic of material that has been subjected to particle size reduction by ball-milling and most other similar industrial procedures employed to reduce particle size (Herdan, 1960). Following sieving several of the particle size ranges were bulked to provide

Sieve Range (μm)	Interval of Range (μm)	Arithmetic Mean of Range (μm)	Geometric Mean of Range (μm)	% In Range By Wt.	Cumulative % Undersize *
> 250	-	-		0.2	99.99
180-250	70	215.0	212.1	3.19	99.79
150-180	30	165.0	164.3	8.39	96.58
125-150	25	137.5	136.9	8.21	88.15
105-125	20	115.0	114.6	21.34	79.91
90-105	15	97.5	97.2	19.32	58.46
75-90	15	82.5	82.2	15.41	39.05
64-75	11	69.5	69.3	10.15	23.56
53-64	11	58.5	58.2	5.97	13.36
38-53	15	45.5	44.9	5.59	7.36
30-38	8	34.0	33.8	1.73	1.75
< 30	-	-	-	0.01	0.01
				Σ 99.51	

* Note. Cumulative % undersize calculated as a % of the final weight of material (99.51 g).

Table 2.4.1 Particle Size Distribution of SSA as Determined by Sieving.

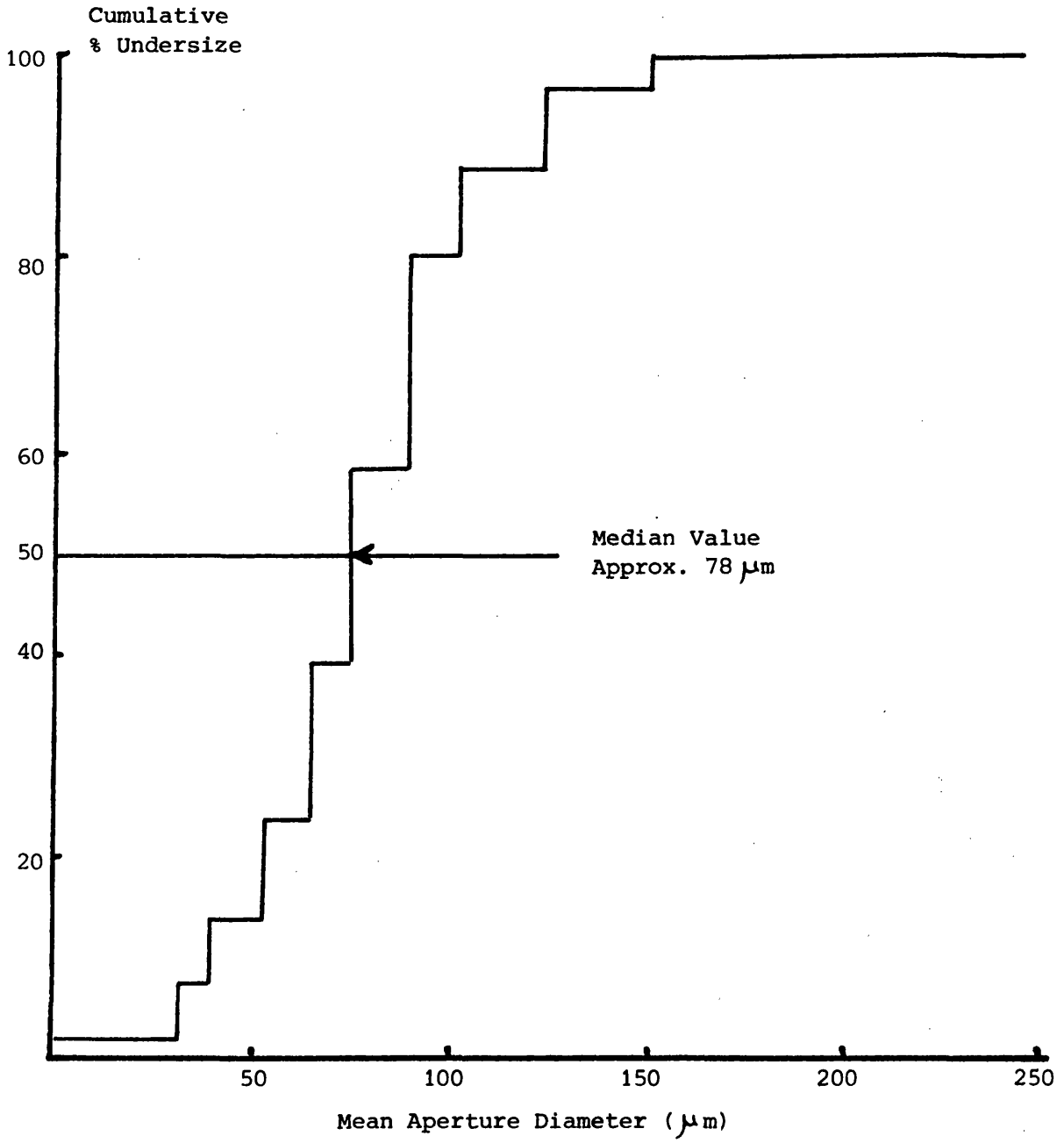


Fig. 2.4.1 Cumulative % Undersize vs Mean Sieve Aperture Diameter.

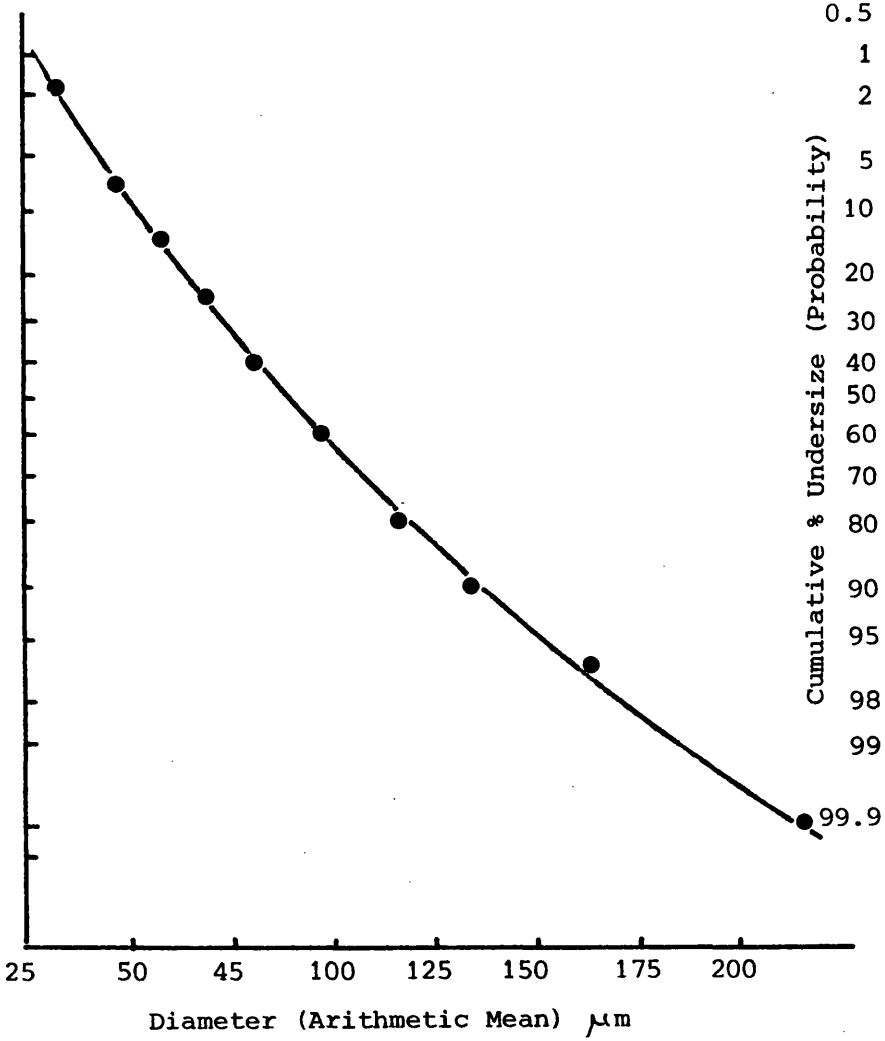


Fig. 2.4.2

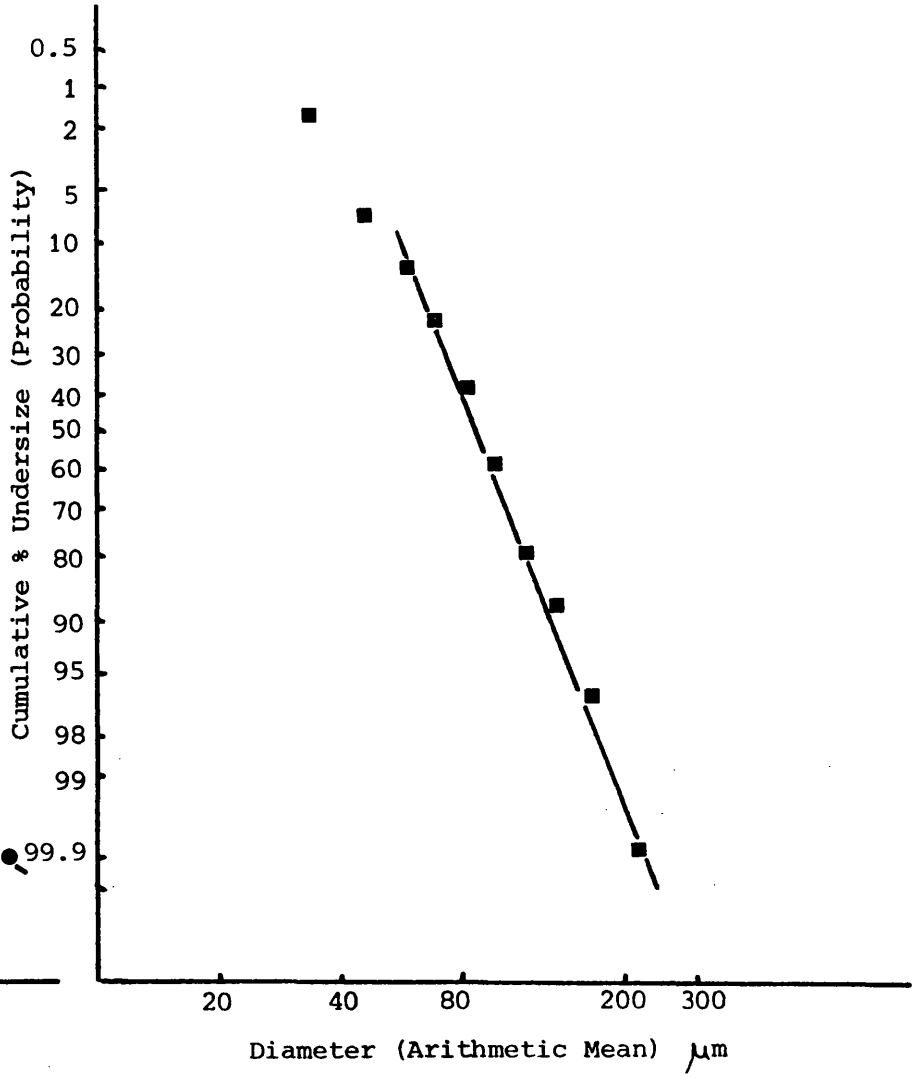


Fig. 2.4.3

Plots of Cumulative % Undersize (Probability Scale) vs Diameter On A Linear Scale (Fig. 2.4.2) And A Logarithmic Scale (Fig. 2.4.3).

sufficient material for all of the proposed in vitro and in vivo studies requiring material of a specific particle size range, and approximately 50 g of subsieve material was "micronised" in a fluid energy mill. Therefore, from this stage onwards the SSA was of the following particle size ranges:- "Micronised"; 30-38; 38-53; 53-75; 75-105; 105-150; 150-180 and greater than 180 μm.

2.4.2 BET SURFACE AREA

BET surface area determinations were carried out at Coulter Electronics Ltd, Luton, using a Digisorb 2500 Automatic Multigas Surface Area and Pore Volume Analyser. Each of the particle size ranges examined was degassed for 16 hours at 45-50^{o} and at a pressure of < 1 μm Hg. Krypton gas was used for the analysis.

Result. The relationship between particle size and BET surface area is shown in Table 2.4.2 and Fig. 2.4.4.

Particle Size Range (μm)	Average * Particle Size (μm)	BET Surface Area ($m^2\ g^{-1}$)
Micronised	10 (5)	0.7683
30 - 38	34	0.5111
38 - 53	45.5	0.5025
53 - 75	66.9	0.3606
75 - 105	91.7	0.2892
105 - 150	139	0.1156
150 - 180	165	0.1105

* Note The average particle size given is calculated from the relative proportions of the bulked sieve fractions and not the arithmetic mean of the total range.

Table 2.4.2 Relationship Between Particle Size and BET Surface Area.

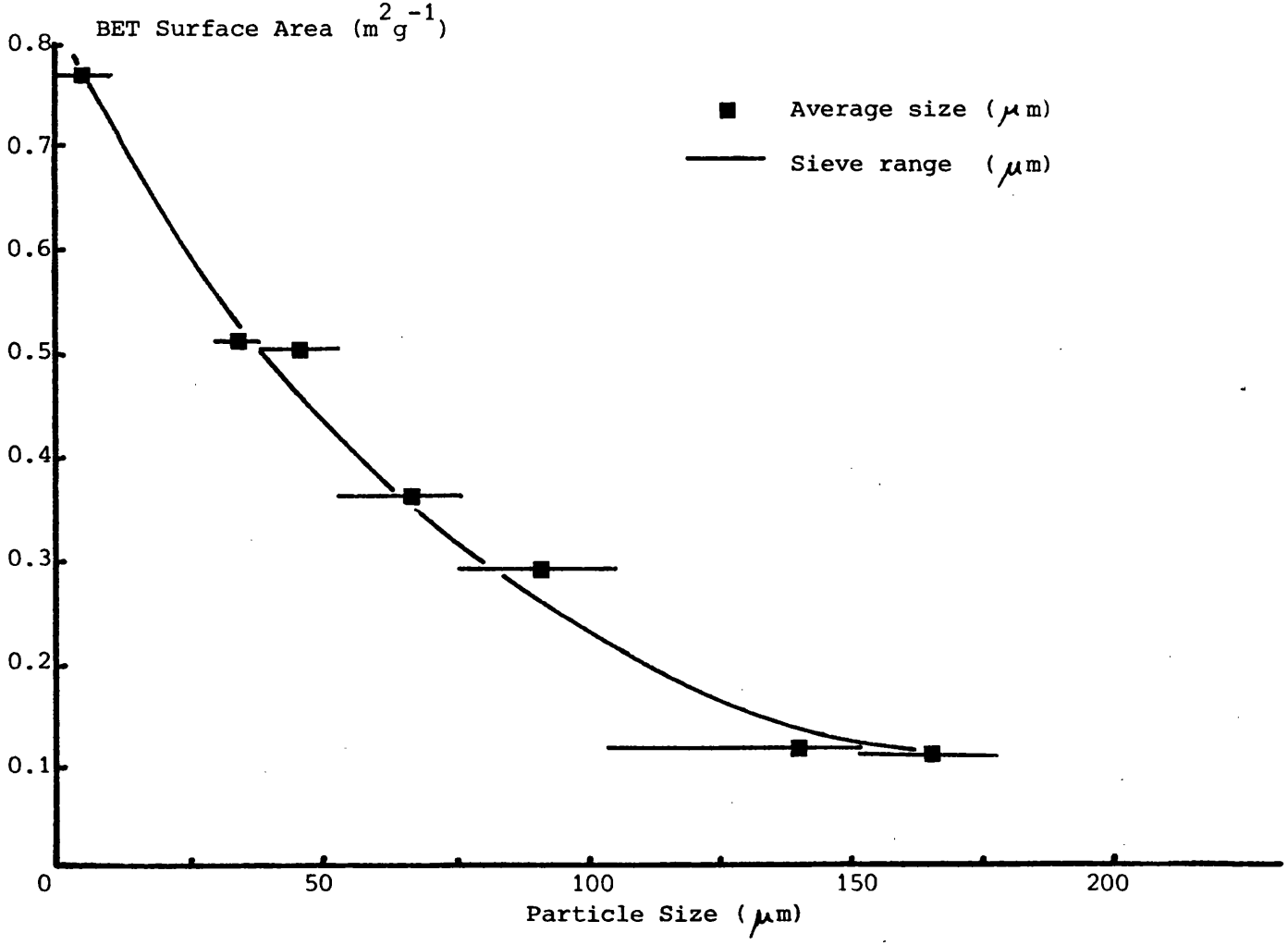


Fig. 2.4.4 Relationship Between Particle Size And BET Surface Area For SSA Sieve Fractions.

2.5 ASSAY OF SSA IN AQUEOUS SOLUTION

The presence of salicylates in solution can be quantitatively measured by many analytical techniques, the majority of which rely on the preliminary conversion of the salicylate to salicylic acid and its subsequent determination by either ultraviolet spectrophotometry, ferric ion chelation followed by visible spectrophotometry, spectrophotofluorimetry, or gas liquid chromatography, (glc), (Kelly, 1970); the most commonly employed methods being the first two cited. A major disadvantage is that, in order to quantitatively measure the parent salicylate, such methods (except glc) rely on a differential assay that monitors salicylic acid concentrations before and after conversion of the parent salicylate to free salicylic acid. Associated with such methodology is the possibility of an increased error that would not occur with direct methods. For this reason a novel assay for SSA was developed, that was both specific and sensitive.

Ultraviolet absorption spectra for SSA and salicylic acid were obtained from their equimolar solutions in 0.1N sodium hydroxide and 0.1N hydrochloric acid containing a final concentration of 1% v/v ethanol. The spectra (Fig. 2.5.1) show a pronounced bathochromic shift for the wavelength of maximum absorption (λ max) of SSA from 310 nm in acid to 341 nm in alkali. The λ max for salicylic acid showed a slight hypochromic change from 307 nm in acid to 301 nm in alkali. The observed bathochromic shift in the λ max for SSA affords a specific method for its measurement in the presence of salicylic acid and quantitative determinations were carried out under alkaline

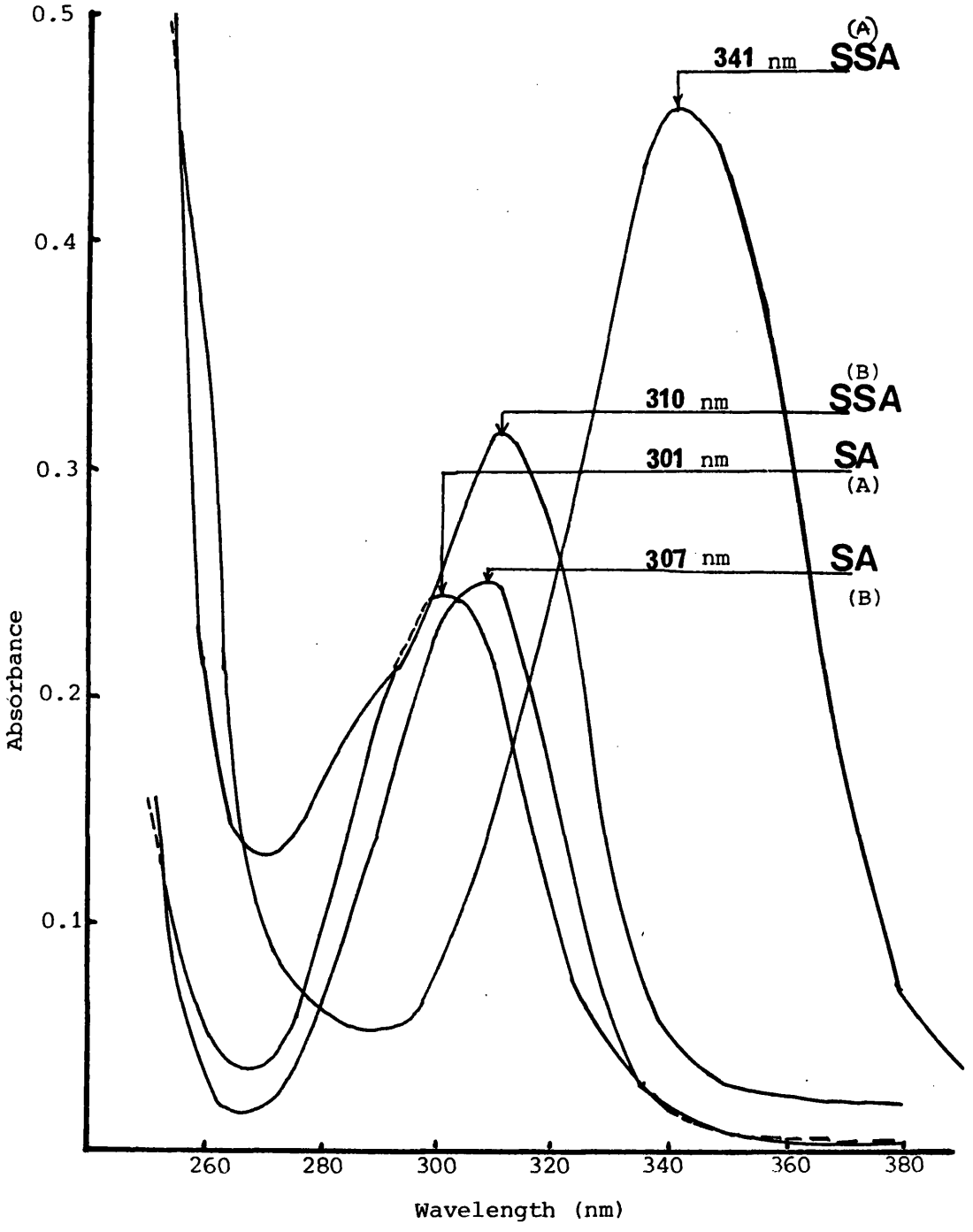


Fig. 2.5.1 Ultraviolet Absorption Spectra For SSA And Salicylic Acid (SA) In 0.1N HCl (B) And 0.1N NaOH (A).

conditions utilising the λ max value at 341 nm.

Adherence to the Beer Lambert Law. A stock SSA solution was prepared by dissolving 100 mg SSA in 1 ml of absolute ethanol in a litre volumetric flask. Sufficient distilled water was rapidly added (to prevent precipitation) and made up to the mark. From this stock solution SSA standards (0.5 to 100 μg ml^{-1}) were prepared by the appropriate dilution in distilled water. 5 ml of each standard was diluted to 10 ml with 0.4N NaOH and the absorbance immediately read at 341 nm against a suitably prepared blank. Preliminary studies showed that the absorbance values obtained in this manner did not change within a two minute period, and the results shown in Table 2.5.1 and Fig. 2.5.2 for duplicate determinations verify that the Beer Lambert Law is obeyed. Comparison of the duplicates by a Student 't' test (Appendix I) shows them not to be significantly different (t_{obs} = 0.263; t_{tab} = 2.12; n = 20; p' = 0.05). The duplicate data was consequently pooled and submitted to linear least-squares regression analysis to provide the equation for the calibration line used in all subsequent SSA determinations in aqueous solution. The absorption coefficient, $E^{1\%}_{1cm}$, and the molar absorption coefficient, ϵ were calculated to be 125.0 and 3226.0 respectively.

2.6 SOLUBILITY OF SALICYLSALICYLIC ACID IN AQUEOUS SOLUTION

Experiments were performed to determine the saturation solubility of SSA in distilled water, 0.1N HCl and their 1% ethanolic solutions.

SSA Concentration ($\mu g.ml^{-1}$)	Absorbance of SSA at 341 nm		
	A	B	
100	1.260	1.262	
80	0.990	1.000	
50	0.636	0.636	
30	0.398	0.401	
10	0.135	0.135	
8	0.109	0.111	
5	0.061	0.060	
3	0.045	0.043	
1	0.019	0.020	
0.5	0.009	0.009	Analysis of Pooled Data
Slope	1.247×10^{-2}	1.250×10^{-2}	1.250×10^{-2}
$\pm$ SE Slope	8.413×10^{-5}	7.721×10^{-5}	5.449×10^{-5}
r	0.9998	0.9998	0.9998
Intercept	7.63×10^{-3}	7.498×10^{-3}	7.563×10^{-3}
$\pm$ SE Intercept	3.762×10^{-3}	3.453×10^{-3}	2.437×10^{-3}

Table 2.5.1 Verification of Beer Lambert Relationship for SSA In 0.1% v/v Aqueous Ethanol at 341 nm

(r is the Correlation Coefficient).

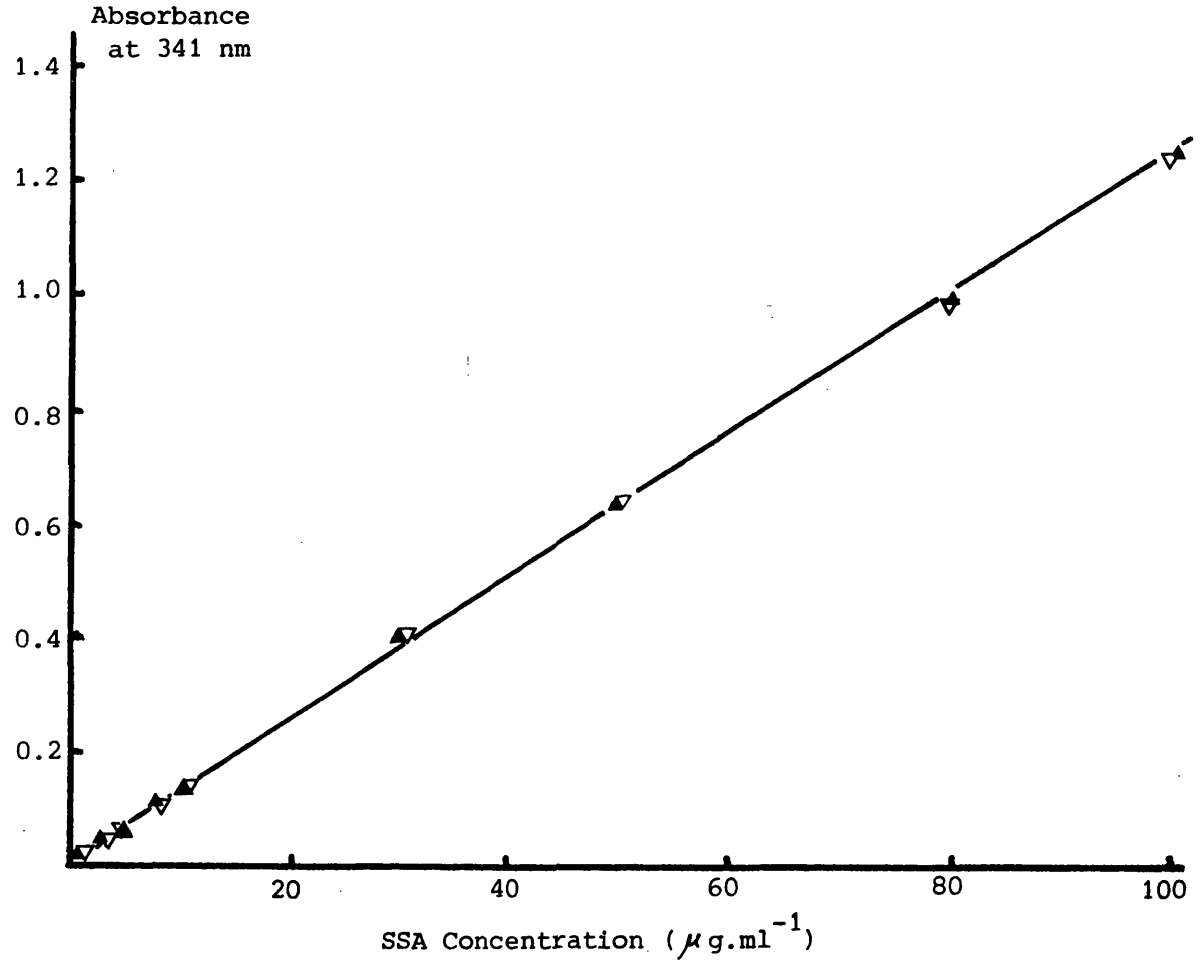


Fig. 2.5.2 Duplicate Beer-Lambert Calibration Curves For SSA Absorbance At 341 nm.

Apparatus. The apparatus (Fig. 2.6.1) consisted of a 250 ml two necked round-bottomed flask placed in a constant temperature ($\pm$ 0.05°) waterbath. Through one neck was inserted a pH electrode, the other neck was used to remove samples for assay. The solution was rapidly stirred by means of a teflon-coated magnetic bar rotated by a submerged magnetic stirrer (Rank Bros., Bottisham, Cambridge).

Initial solubility determinations were very irreproducible due to inadequate separation of the solid material from the liquid phase by the sintered-glass tubes originally employed for that purpose. Furthermore, difficulty was encountered in the use of sintered-glass filters of smaller pore size. Removal of undissolved material was efficiently achieved by filtration through a 0.45 μm membrane filter (Millipore, London) held in a 25 mm Swinnex membrane filter holder (Millipore, London) that had been adapted to two-directional flow. This backwash facility was essential for mixing of the dead spaces in the sample tube and the membrane holder. Removal of filtrate into a 10 ml syringe allowed subsequent accurate pipetting of the sample prior to assay. This procedure for separation of undissolved particulate matter was used in all experiments requiring a similar type of filtration prior to assay (eg. SSA recrystallisation and dissolution studies).

Method. Excess material was added to 100 ml of solvent (previously equilibriated to the required temperature) and rapidly stirred. Approximately 6 ml samples were removed at intervals, from which 5 ml was pipetted, diluted if necessary,

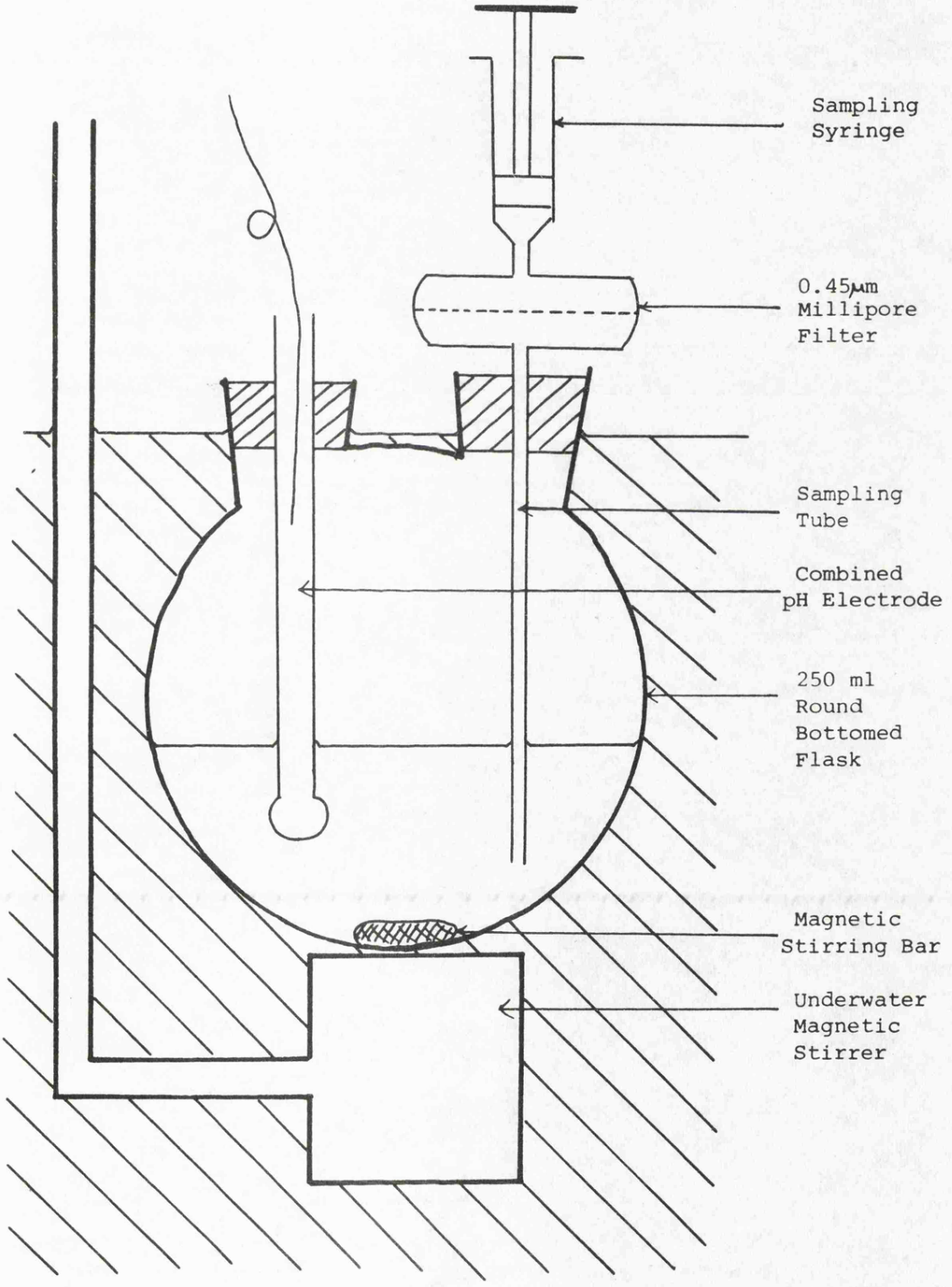


Fig. 2.6.1 Solubility Apparatus

and assayed. The concentration was corrected for changes in volume due to temperature and monitored until no further increase was observed. Table 2.6.1 and Fig. 2.6.2 show the relationship between saturation solubility and temperature (expressed as a van't Hoff plot) for SSA in water, 0.1N HCl and their 1% v/v ethanolic solutions.

Temperature		Equilibrium Concentration of SSA ($\mu g\ ml^{-1}$)			
($^{\circ}C$)	$^{1}/_{T}$ ($X10^{-3}K$)	Distilled Water ± SE	1% v/v Ethanol ± SE	0.1N HCl ± SE	0.1N HCl +1% v/v Ethanol ± SE
19.85	3.415	88.82 ± 0.27	93.34 ± 0.55	37.47 ± 0.26	42.86 ± 0.94
29.60	3.305	119.96 ± 0.74	128.61 ± 1.06	56.07 ± 0.54	67.65 ± 0.29
40.05	3.194	168.85 ± 2.90	177.39 ± 1.99	92.02 ± 2.13	96.87 ± 0.95
50.00	3.096	240.99 ± 1.30	257.49 ± 1.38	144.24 ± 1.10	155.77 ± 0.66
59.95	3.003	353.24 ± 3.32	380.87 ± 7.01	239.36 ± 2.42	264.77 ± 4.58
69.60	2.919	536.81 ± 18.37	585.42 ± 8.37	411.93 ± 4.33	432.20 ± 3.43

Table 2.6.1 Influence of Temperature on the Equilibrium Solubility of SSA in Distilled Water, 0.1N HCl and Their 1% Ethanolic Solutions.

The van't Hoff plot for SSA solubility exhibits a curvilinear profile that precludes the determination of heats of solution. What is evident is that 1% v/v ethanol increased the solubility of SSA in both distilled water and 0.1N HCl by approximately

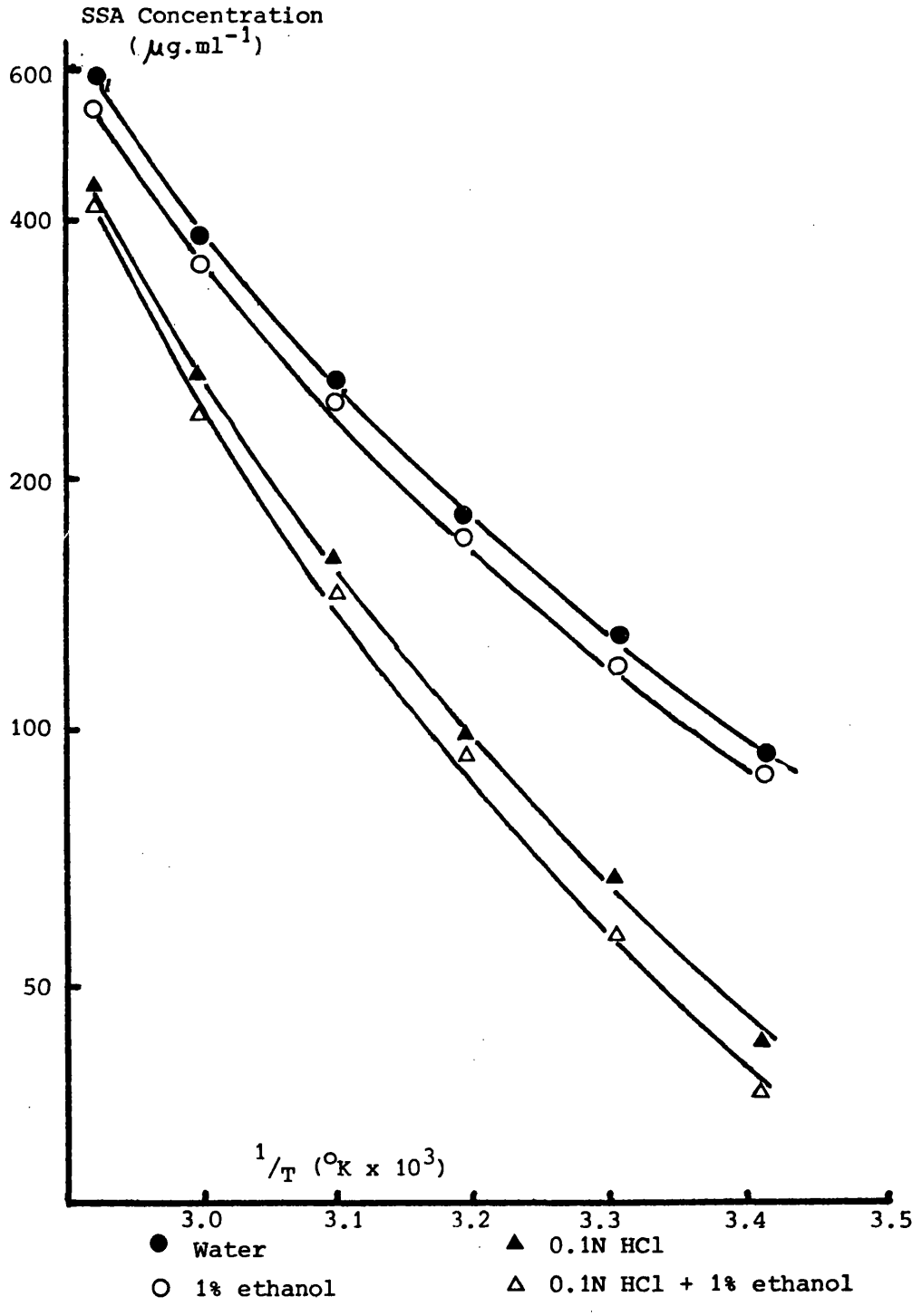


Fig. 2.6.2 Saturation Solubility Of SSA In Water 0.1N HCl And Their 1% Ethanolic Solutions With Respect To Temperature.

the same order of magnitude (1.068 and 1.109 respectively). A plot of solubility ratio (solubility in 0.1N HCl/distilled water) vs temperature is shown in Fig. 2.6.3 and illustrates that SSA solubility increases to a greater extent in 0.1N HCl than in distilled water. The data used to provide Fig. 2.6.3 is taken from Table 2.6.2 and calculated from that in Table 2.6.1.

Temperature (°C)	Solubility Ratio 0.1N HCl/Distilled Water
19.85	0.421
29.60	0.467
40.05	0.545
50.00	0.599
59.95	0.678
69.60	0.767

Table 2.6.2 Influence of Temperature on the 0.1N HCl/ Distilled Water Solubility Ratio of SSA.

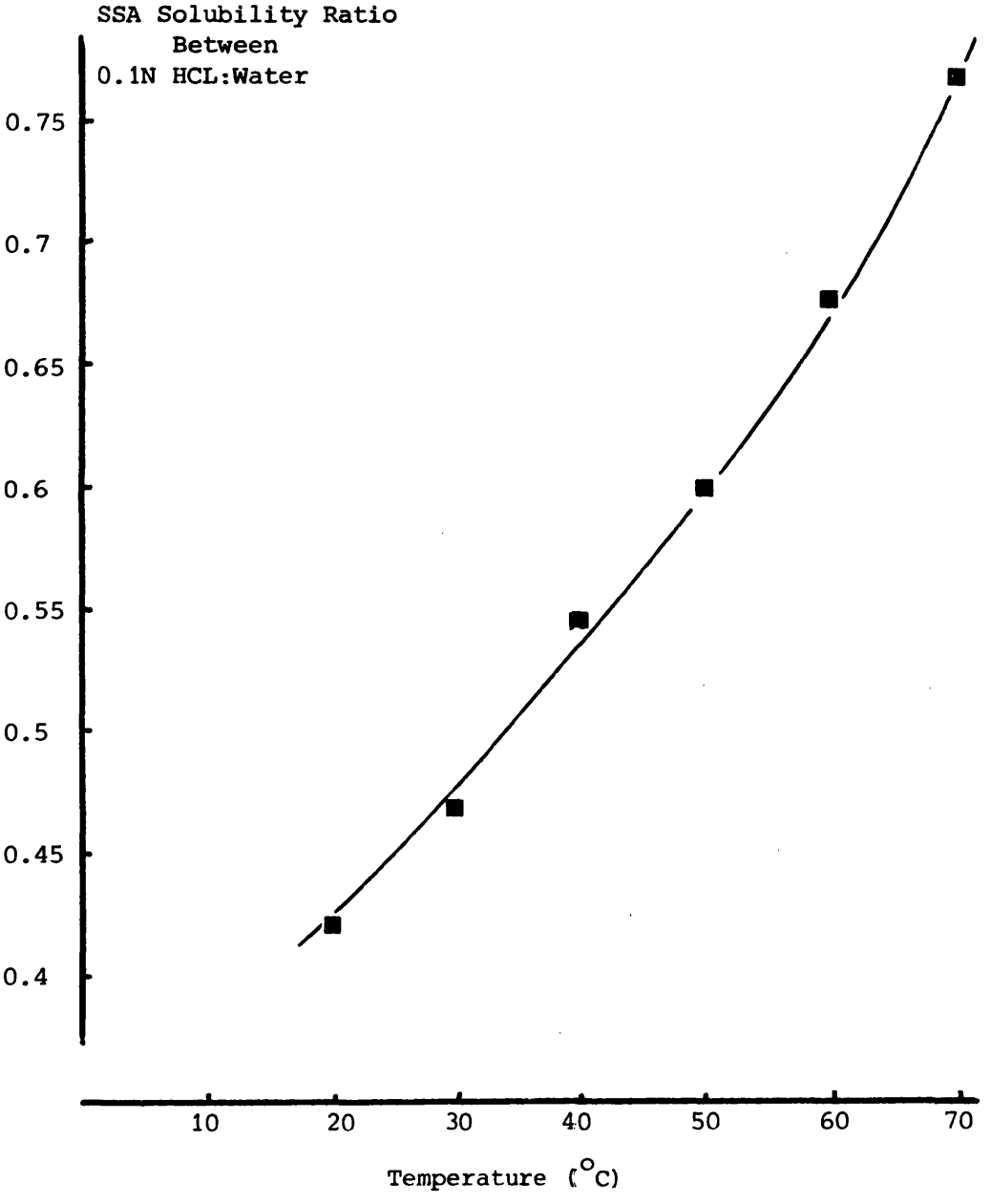
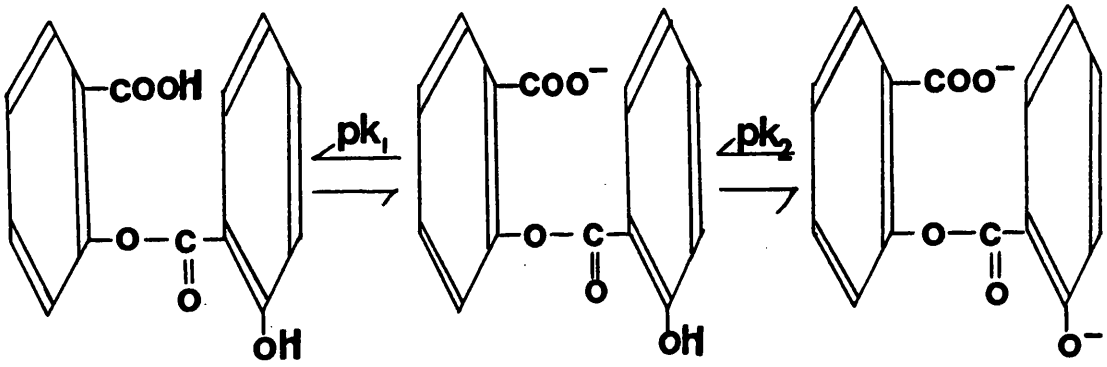


Fig. 2.6.3 Influence Of Temperature On 0.1N HCl/Distilled Water Solubility Ratio Of SSA.

2.7 DETERMINATION OF IONISATION CONSTANTS OF SSA.

SSA may be expected to ionise according to the following scheme:



Two methods were employed to determine the pK_1 and pK_2 values at 25°.

2.7.1 POTENTIOMETRIC TITRATION

Owing to the poor solubility of SSA a saturated solution was titrated against 0.02N NaOH. Mixing was achieved by a magnetic stirring bar and each titration was carried out in vessels immersed in a thermostatically controlled waterbath at 25 ± 0.1°.

The titration curves produced are typified by the example shown in Fig. 2.7.1. In order to accurately interpolate the equivalence point a plot of the rate of change of pH with respect to volume of titre ($^{dpH}/_{dv}$) is also given. It is clear from the curve that it was not possible to exactly identify the equivalence point, therefore pK_a determination by this method was abandoned.

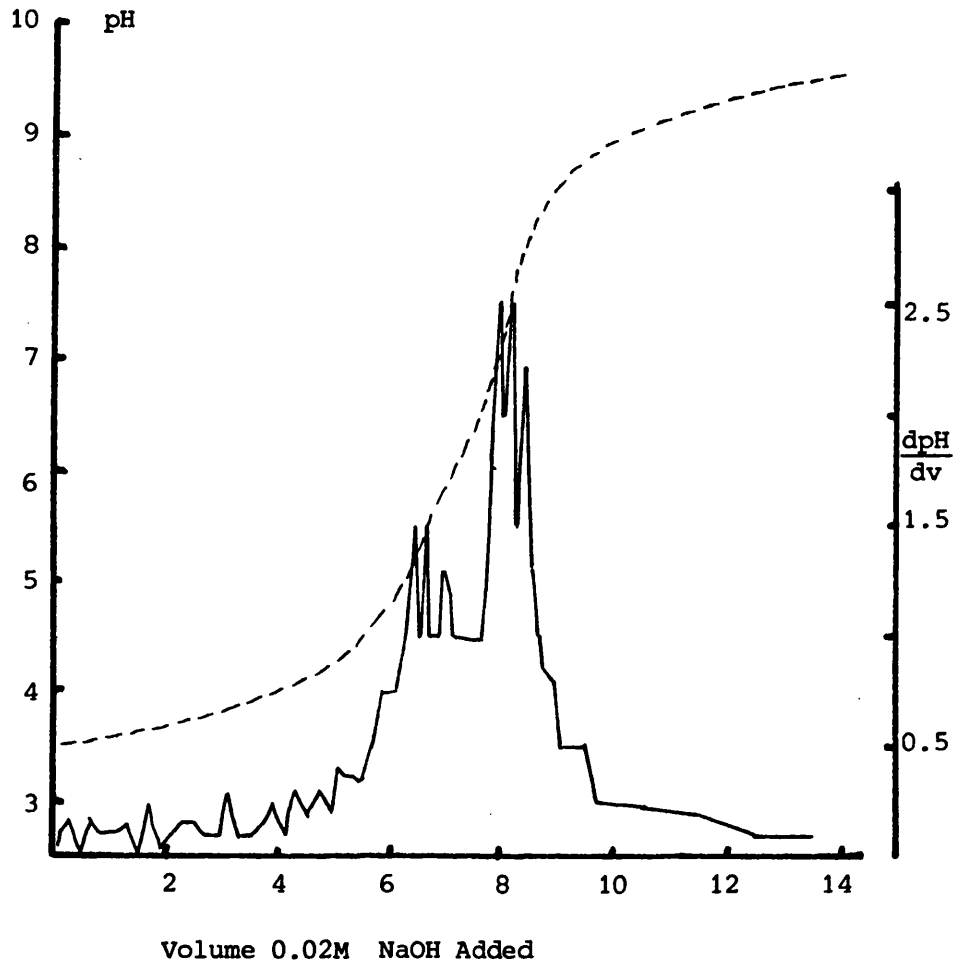


Fig. 2.7.1 Potentiometric Titration Curve (---) for SSA (Saturated Solution) And Derived Rate Of Change Of pH vs Volume Of Titre $\left(\frac{dpH}{dv}\right)$ (Solid Line) at 25°.

2.7.2 ULTRAVIOLET SPECTROPHOTOMETRY

The bathochromic shift observed previously (see Fig. 2.5.1) indicated that ultraviolet spectrophotometry may be suitable to determine pK_2, it was further anticipated that there might also be a spectral change suitable for pK_1 determination. The theoretical basis for determination of ionisation constants by ultraviolet spectrophotometry is given in Appendix II.

Method. The ultraviolet absorption spectra of SSA with respect to pH were examined using drug solutions in the buffers set out in Table 2.7.1.

Buffer	pH Range
Clark and Lubs KCl:HCl 0.2N	1.0 - 2.2
McIlvaines citrate:phosphate	2.2 - 5.5
Sørensens phosphate	5.0 - 8.0
Gomoris' tris:HCl	7.2 - 9.0
Sørensens glycine:NaOH	8.4 - 13.0

Table 2.7.1. Buffer Systems Employed In pK_a Determinations. (Documenta Geigy, 1962).

All buffer solutions were prepared double strength. A stock SSA solution containing approximately 70 $\mu g.ml^{-1}$ (accurately known) in 1% v/v ethanol was prepared and equilibrated at $25 \pm 0.1^{\circ}$ with the buffer solutions. After equilibration each buffer solution was diluted with an equal volume of SSA stock solution and the absorbance spectra obtained with reference to

the appropriately treated buffer using a constant temperature cuvette housing (Unicam SP874). For high pH values the SSA spectra did not show any significant changes within a five minute period after preparation, nevertheless spectra were always obtained immediately after preparation of SSA solutions and the pH was measured during the recording of the spectra. To establish the potential of ultraviolet spectrophotometry in the determination of SSA ionisation constants spectra were recorded at 0.5 pH intervals throughout the range pH 1.0-13.0. The extremes of this range were produced in 0.1N HCl and NaOH respectively.

Examination of the resultant spectra, examples of the most important ones are given in Fig. 2.7.2 show the following changes with respect to pH.

pH 0.65 - 5.00	A shoulder to the main peak exists at 285 nm which decreased with increasing pH.
pH 5.00 - 8.00	No change in spectra occurred.
pH 8.00 - 11.50	An increased absorbance associated with a change in λ max from 310 nm to 341 nm (Bathochromic shift).
pH 11.50 - 13.00	No change in spectra occurred.

Superimposed plots of absorbance at 285, 310 and 341 nm against pH are shown in Fig. 2.7.3.

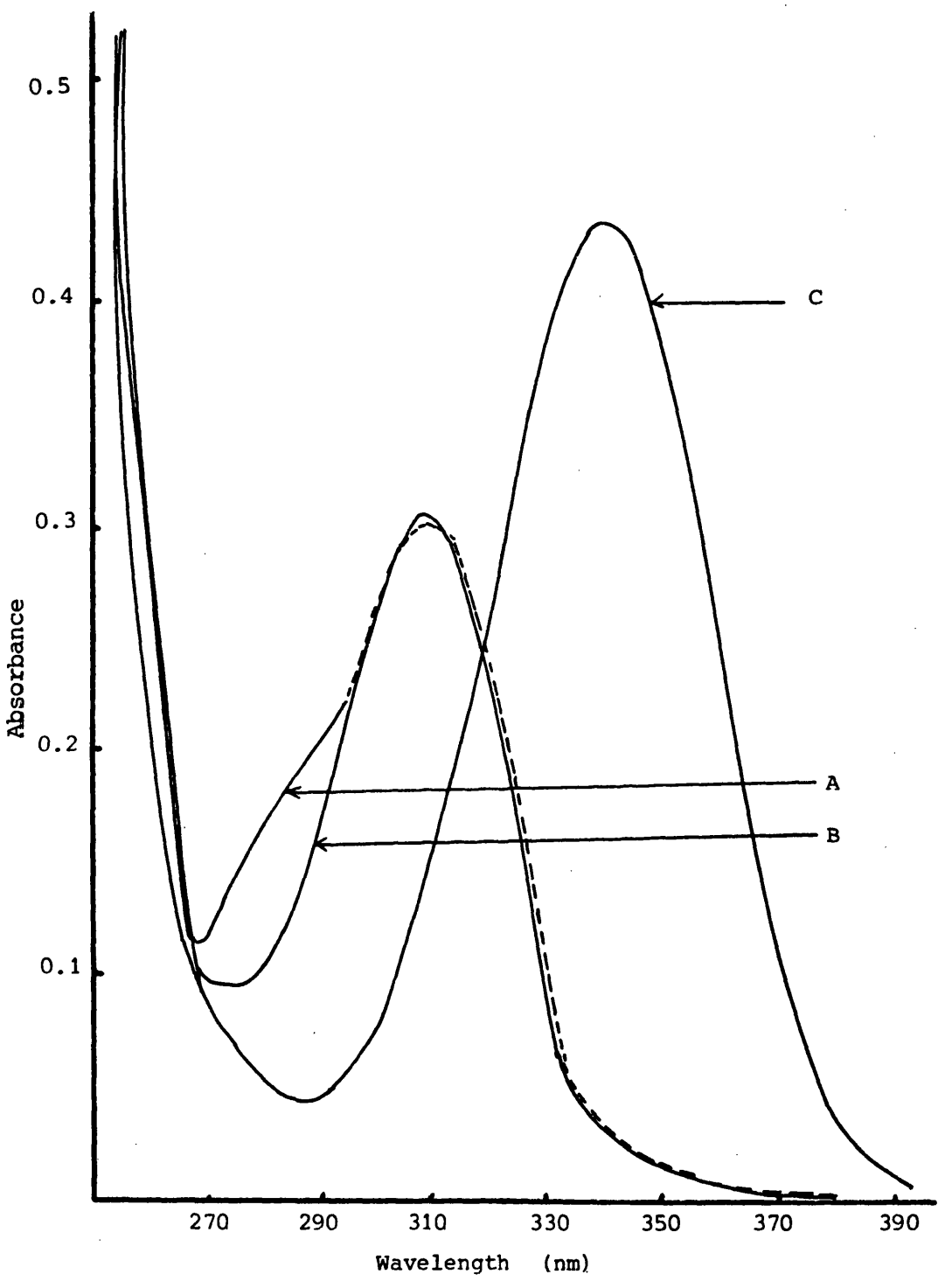


Fig. 2.7.2 Ultraviolet Absorption Spectra For SSA At pH 0.65 (A), 5.00 (B) And 12.25 (C)

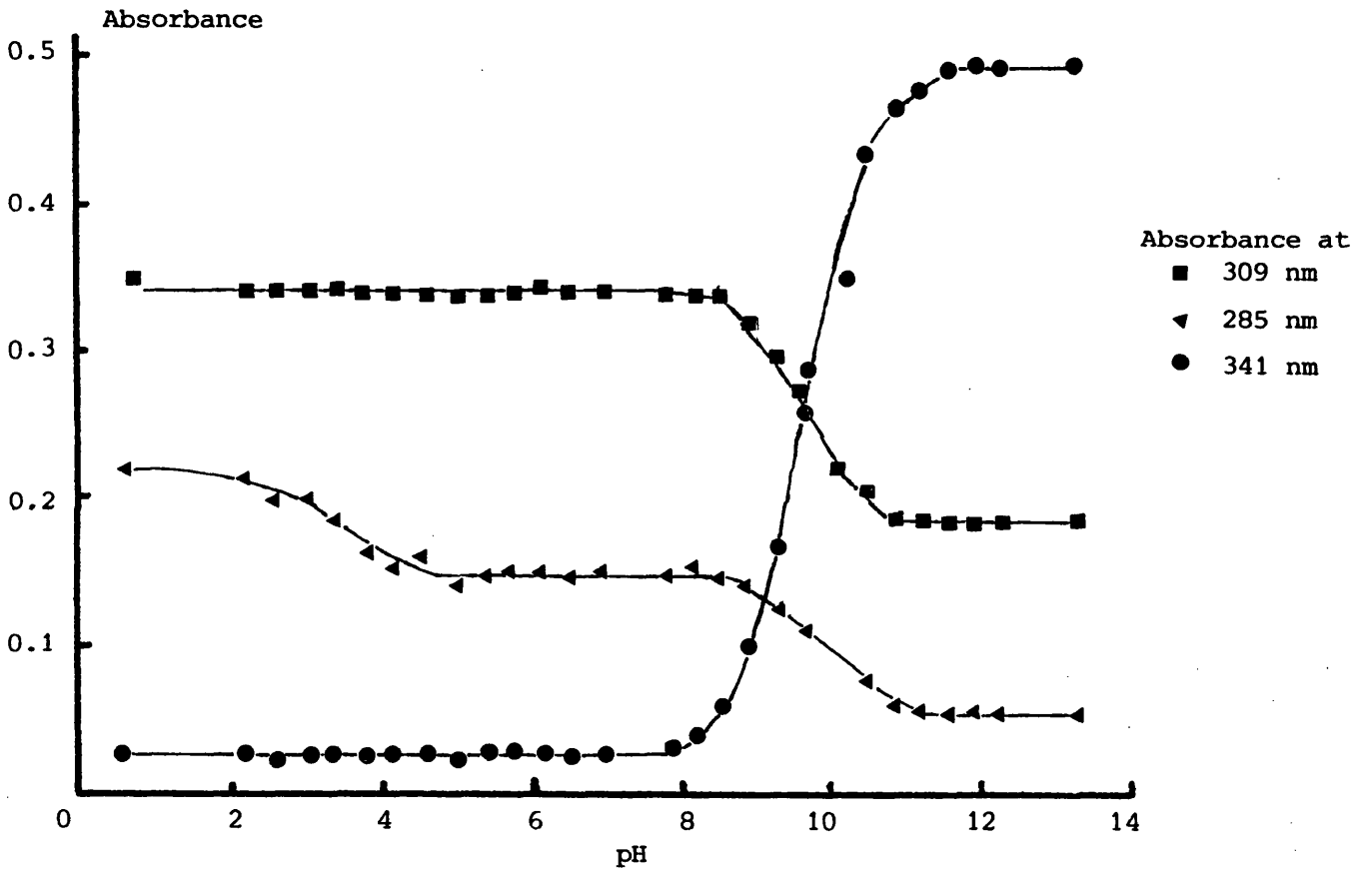
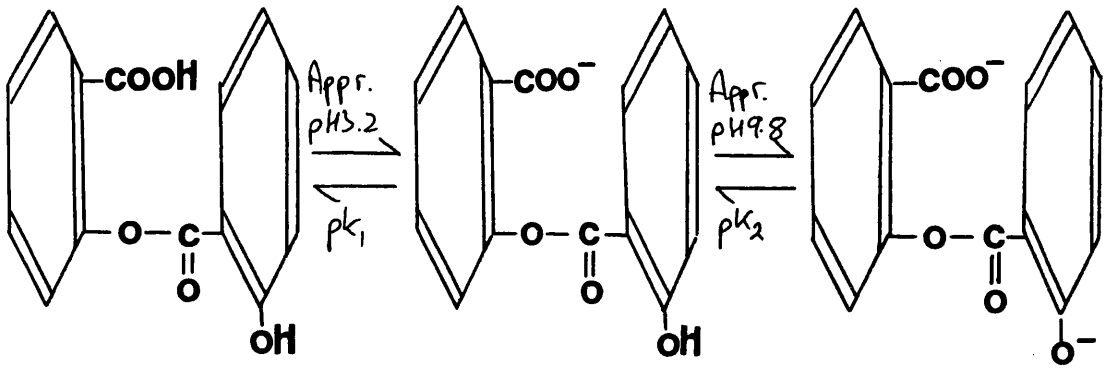


Fig. 2.7.3 Absorbance vs pH For SSA (approx. 38.5 $\mu g.ml^{-1}$) At 285, 309 And 342 nm.

From these plots the following scheme for SSA ionisation can be made:-



and approximate pK values obtained by interpolation from Fig. 2.7.3. are pK_1 = 3.2 and pK_2 9.8.

Subsequent determinations, to define the pK_a values, were performed within the pH ranges 0.65 - 5.0 and pH 8.0 - 13.0 for pK_1 and pK_2 values respectively, using "analytical wavelengths" of 285 and 341 nm. Double strength buffer solutions were prepared at 0.2 pH unit intervals within those ranges, the final pH of the diluted test solution was again measured at 25° while the absorbance was measured.

Result. Using equation (63) in Appendix II, the pK_1 and pK_2 were calculated from a knowledge of the measured pH, the absorbance at that pH and the absorbance of the unionised and ionised species for that ionisation. From triplicate determinations of pK_1 and pK_2, the data for which is given in Table 2.7.2, the respective values are:-

$$pK_1 = 3.450 \pm 0.008$$

$$pK_2 = 9.825 \pm 0.003 \text{ at } 25^{\circ}.$$

	pK_1			pK_2		
	A	B	C	A	B	C
	3.4108	3.4775	3.4170	9.8080	9.8303	9.8173
	3.3928	3.4509	3.4771	9.8370	9.8351	9.8185
	3.3703	3.4558	3.4750	9.8311	9.8150	9.8374
	3.4599	3.4380	3.4904	9.8331	9.8091	9.8068
	3.4053	3.4778	3.4178	9.8420	9.8213	9.8019
	3.4278	3.5075	3.4429	9.8369	9.8238	
	3.4628	3.4559	3.4066	9.8546	9.8108	
		3.5528				
		3.4626				
		3.4608				
$\bar{x}$	3.4195	3.474	3.447	9.835	9.821	9.816
SD	0.034	0.034	0.035	0.010	0.010	0.016
SE	0.013	0.011	0.013	0.004	0.004	0.007
Grand Mean	3.450 ± 0.008			9.825 ± 0.003		

Table 2.7.2 Triplicate Determinations of pK_1 And pK_2 For SSA at $25 \pm 0.1^{\circ}C$

2.8 PARTITION COEFFICIENT

If a substance is added to a system composed of two immiscible solvents, in an amount insufficient to saturate either phase, it will become distributed between the two phases in a definite concentration ratio. This ratio (K), also known as the distribution ratio, distribution coefficient or partition coefficient, can be expressed by the simple form of the Distribution Law:-

$$K = \frac{C_1}{C_2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (19)$$

where C_1 and C_2 are the equilibrium concentrations of the substance in solvents 1 and 2 respectively. There is no established convention as to whether the partition coefficient is expressed as C_1/C_2 or vice versa; consequently the respective phases are stated, either generally (eg. oil/water), or specifically (eg. octanol/water).

The simple form of the Distribution Law only applies to dilute solutions where a single molecular species is common to both phases. Hence when association occurs in either phase or ionisation occurs in the aqueous phase the overall distribution is more complex. In the latter case the total concentration of an acidic drug in the organic phase (C_o), represents the unionised concentration ($[HA]_o$), since ionisation does not occur in that phase. The true partition coefficient

$$K = \frac{[HA]_o}{[HA]_w} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (20)$$

where $[HA]_o$ and $[HA]_w$ are the concentrations of the unionised form in the oil and aqueous phases respectively. However, the total drug concentration in the aqueous phase,

$$C_w = [HA]_w + [A^-]_w \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (21)$$

where $[A^-]_w$ is the aqueous concentration of the ionised species. Further, if the total drug concentration in the aqueous phase is employed in the determination of the distribution between the two phases then the experimentally observed, or apparent, partition coefficient K' is given by:-

$$K' = \frac{C_o}{C_w} = \frac{[HA]_o}{[HA]_w + [A^-]_w} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (22)$$

The true partition coefficient can be obtained by extrapolation from apparent partition coefficients determined over a range of hydrogen ion concentrations (See Martin et al, 1970), or by analysis of the oil phase and of the water phase at a sufficiently low pH (ie pH = pK_a-2) where 99% of the drug would exist in the unionised form. The latter method was employed here since solutions of SSA prepared in 0.1N HCl ensure that the drug is 99.6% unionised.

Method. Prior to use 1-octanol was presaturated with 0.1N HCl at 25°. A solution of SSA (approximate concentration 50 $\mu g.ml^{-1}$) was prepared in 0.1N HCl. Six 20 ml volumes of solution were shaken with an equal volume of presaturated 1-octanol in 50 ml conical flasks (Quickfit) in a shaking waterbath (Type SS30 Grant Instruments, Cambridge) at 24.5 $\pm$ 0.1°C for 16 hours; a time that had been shown to be sufficient for equilibrium to be

achieved. The shaking rate was approximately 120-150 min^{-1} and did not produce any emulsification within the system. After shaking the flasks were allowed to stand for one hour for the two phases to separate. Five ml of the aqueous layer was removed by pipette through the 1-octanol. Care was taken to prevent the organic solvent from entering the pipette, similarly, 1-octanol was removed from the exterior of the pipette with a tissue. The initial and final SSA concentrations of the aqueous phase were assayed as described previously (See 2.5) and the drug concentration in the 1-octanol calculated by difference. 1-octanol was shown not to interfere with the assay. The pH of the aqueous solution was checked before and after shaking and was found not to alter from pH 1.02. Table 2.8.1 shows the final concentrations of SSA for the 0.1N HCl and 1-octanol phases.

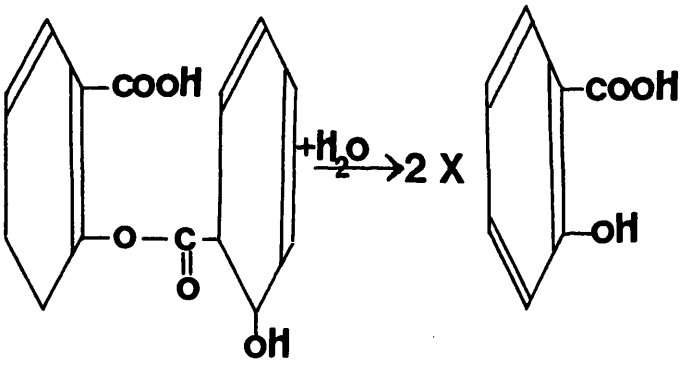
Sample	Absorbance at 341 nm	SSA $\mu g.ml^{-1}$ 0.1N HCl	1-octanol
Initial	0.593	47.44	0
1	0.006	0.48	46.96
2	0.006	0.48	46.96
3	0.017	1.36	46.08
4	0.006	0.48	46.96
5	0.023	1.84	45.60
6	0.020	1.60	45.84
	Mean	1.04	46.40

Table 2.8.1 Partitioning of SSA Between 1-octanol and 0.1N HCl at 24.5°C.

From Table 2.8.1 a 1-octanol/0.1N HCl partition coefficient of 44.62 is obtained, and demonstrates that the unionised form of SSA is highly lipid soluble and suggests that the unionised species of SSA will be freely permeable across the gastrointestinal membranes.

2.9 DETERMINATION OF THE STABILITY OF SSA

SSA can be expected to hydrolyse in aqueous solution according to the following scheme.



In order to follow the time course of the hydrolytic reaction it was necessary to quantitatively differentiate between SSA and salicylic acid.

2.9.1 ASSAY FOR SSA IN THE PRESENCE OF SALICYLIC ACID

Examination of Fig. 2.5.1 shows that SSA and salicylic acid can be differentiated by examination of the ultraviolet absorption spectrum in solutions at high pH as previously demonstrated (See 2.5). However it was essential to ensure that the sodium hydroxide used to induce spectrum changes was of sufficient strength to cope with all the buffer systems (Table 2.9.1) used in these studies.

A solution of SSA ($\simeq 37.5\,\mu g.ml^{-1}$) was prepared in buffer systems throughout the range pH 1.0 - 13.0. The absorbance at 341 nm and the pH of each SSA buffer system were measured before and after dilution with equal parts of 0.4N NaOH. Since hydroxyl and hydronium ions can catalyse hydrolytic degradation it was necessary that the absorbance values were obtained immediately after alkaline dilution. Table 2.9.1 shows that such alkaline dilution was sufficient to raise the pH of each buffer system to a value greater than pH 12.0.

Buffer System	Measured pH of SSA:Buffer		Absorbance at 341 nm	
	Before Dilution	After Dilution	Before Dilution	After Dilution
Clark and Lubs KCl:HCl	1.0	12.80	0.035	0.477
McIlvaines	2.94	12.60	0.036	0.475
Citric Acid:	5.00	12.60	0.034	0.478
Phosphate	7.00	12.60	0.037	0.478
Sørensens	8.80	12.80	0.114	0.474
Glycine:NaOH	10.82	12.90	0.835	0.472
	12.78	13.00	0.940	0.472

Table 2.9.1 Influence of 0.4 NNaOH on the pH and Absorbance of SSA (37.5 $\mu g.ml^{-1}$) in Different Buffer Systems

Salicylic acid influences on the absorbance spectrum of SSA are evident on examination of Fig. 2.5.1 which shows salicylic acid to have a low absorbance in alkaline media at 341 nm and should therefore be accounted for in the assay of SSA.

For a two component system where both components absorb at a given wavelength the Beer-Lambert Law has to be modified from equation (53) in Appendix II to (23).

$$A = \epsilon_1 c_1 + \epsilon_2 c_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (23)$$

where A is the absorbance at a given wavelength and ϵ and c are the molar absorption coefficient and concentration respectively for components 1 and 2. The hydrolysis of 1 mole of SSA would result in the production of 2 moles of salicylic acid. If the fraction of SSA (initial molar concentration) remaining after time, t, is given by x, then the fraction of salicylic acid produced will be 2(1-x) and therefore from equation (23)

$$A = A_1 x + 2A_2 (1-x) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (24)$$

$$= A_1 x + 2A_2 - 2A_2 x \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (25)$$

where A is the absorbance of the two component mixture and A_1 and A_2 are the absorbances of SSA and salicylic acid respectively. It follows from equation (25) that

$$x = \frac{A - 2A_2}{A_1 - 2A_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (26)$$

The validity of this reasoning was established by measurement of the absorbance (at 341 nm) of eight standard solutions of SSA (in glycine buffer pH 12.5) before and after complete hydrolysis. Hydrolysis was achieved by autoclaving the standards at a pressure of 15 lb in^{-2} for three hours. The post-autoclaving

absorbances were compared to eight salicylic acid standards of twice the equivalent SSA molar concentration, similarly prepared in glycine buffer at pH 12.5. The results in Table 2.9.2 and Fig. 2.9.1 indicate that complete SSA degradation to salicylic acid had occurred, and that the Beer-Lambert plot obtained for the hydrolytic products was superimposable and insignificantly different ($t_{obs} = 0.969$; $t_{tab} = 2.18$; $n = 16$; $p' = 0.05$) from that for equivalent solutions of salicylic acid.

The % residual concentration for SSA can therefore be calculated using equation (27)

$$\% \text{ residual concentration} = \frac{A_t - A_\infty}{A_o - A_\infty} \times 100 \quad \ldots\ldots\ldots \quad (27)$$

where A_t is the absorbance at time, t, A_∞ is the absorbance at time, $t = \infty$ (ie after complete hydrolysis), and A_o is the absorbance at time, t = o.

2.9.2 GENERAL METHOD

50 ml of SSA (approximately 75 $\mu g.ml^{-1}$) solution prepared in 0.1% v/v ethanol and double strength buffer of the appropriate pH were equilibriated to the required temperature in separate vessels. At time, t = o, they were mixed and a zero hour 5 ml sample taken, further samples of 5 ml were removed and assayed for SSA according to the method described (See 2.5). Duplicate experiments were performed in all cases and the initial and final pH values for each experiment were determined. The apparent first order rate constants for the hydrolysis of SSA were calculated from log-linear plots of the percentage residual

	After Hydrolysis		Before Hydrolysis				
SSA $\mu g.ml^{-1}$	pH	Abs at 341 nm	pH	Abs at 341 nm	Salicylic Acid $\mu g.ml^{-1}$	pH	Abs at 341 nm
100	12.64	0.087	12.67	1.250	107	12.63	0.096
80	12.68	0.075	12.77	1.020	85.6	12.65	0.078
60	12.61	0.058	12.76	0.750	64.2	12.62	0.062
40	12.58	0.038	12.78	0.532	42.8	12.60	0.042
20	12.60	0.022	12.76	0.255	21.4	12.60	0.024
10	12.62	0.014	12.76	0.127	10.7	12.68	0.012
5	12.69	0.009	12.76	0.073	5.35	12.59	0.006
2	12.60	0.009	12.77	0.028	2.14	12.61	0
Slope		8.340×10^{-4}		1.252×10^{-2}			8.973×10^{-4}
Intercept		0.006		0.008			0.002
SE Slope		1.883×10^{-5}		1.260×10^{-4}			2.375×10^{-5}

Table 2.9.2 Comparison of the Absorbances at 341 nm of Standard Solutions of SSA (Before and After Hydrolysis), With Salicylic Acid

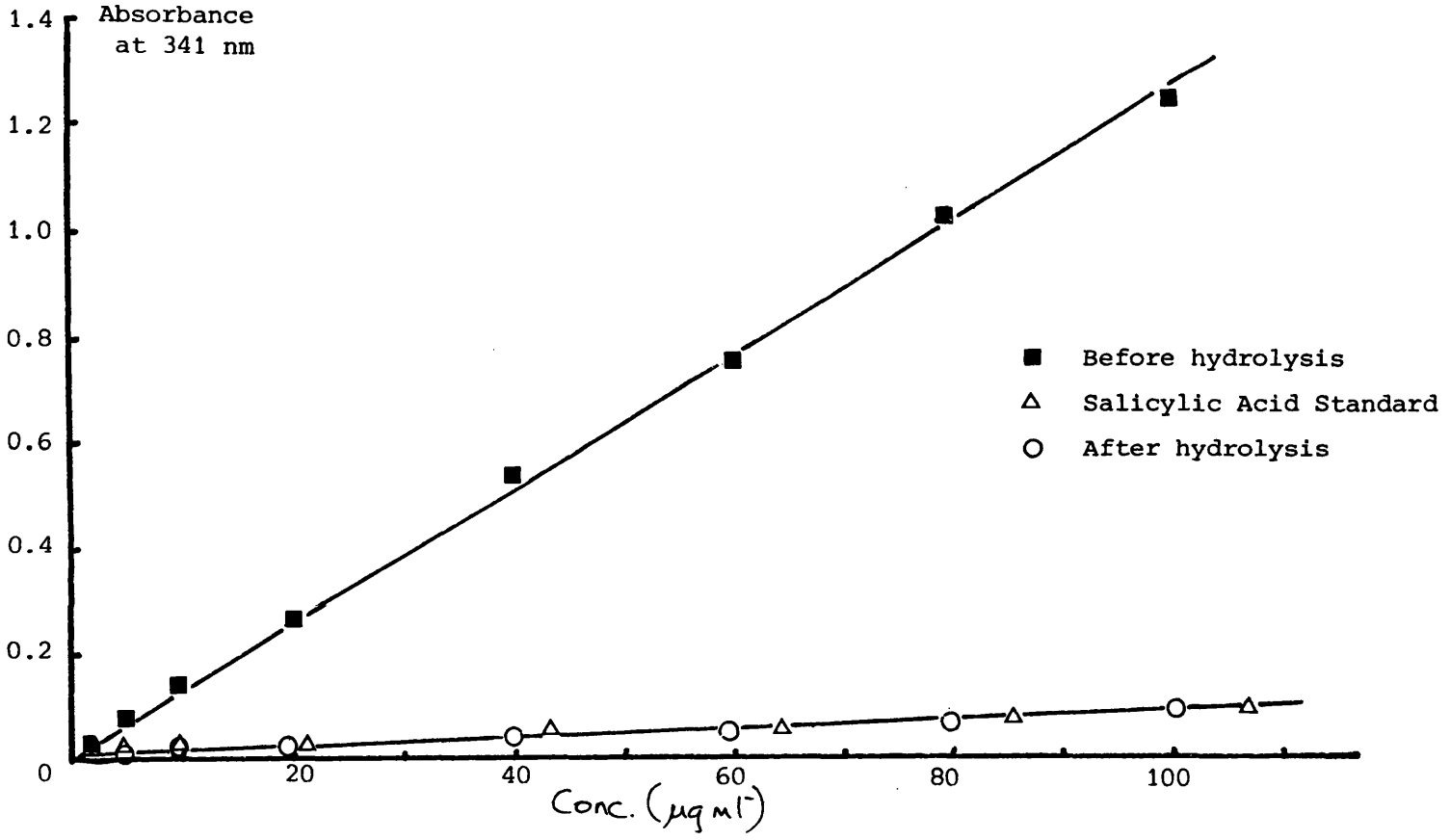


Fig. 2.9.1 Absorbance Of SSA At 341 nm In Glycine Buffer (pH 12.5) Before And After Hydrolysis By Autoclaving At 15 p.s.i. For Three Hours In Comparison To Salicylic Acid Standards.

concentration against time. The values of slope and intercept, together with their respective standard errors, were calculated by submission of the data to linear least-squares regression analysis.

2.9.3 INFLUENCE OF BUFFER AND IONIC STRENGTH

The following buffer systems for the pH range 1.0 - 13.0 were prepared double strength according to Documenta Geigy (1962).

Buffer System	pH Range
Clark and Lubs KCl:HCl	1.0 - 2.2
McIlvaines Citrate:phosphate	2.2 - 8.0
Gomoris tris:HCl	7.3 - 9.0
Sørensens glycine:NaOH	8.5 - 13.0

A comparison of the apparent first order rate constants for SSA hydrolysis in different buffer systems at the same pH was performed at a transition pH from one buffer system to another in single strength buffers at 50^{o}. Fine pH adjustments were made using minimal volumes (less than 0.5 ml) of Normal HCl or NaOH and final concentrations were corrected for temperature related changes in the sample volume.

Ionic strength effects were studied using single and half strength buffers at a pH within the range covered by that system.

The data presented in Table 2.9.3 indicates that no one buffer system significantly influenced the apparent first order rate constant for SSA hydrolysis at a given pH. The data for each pH was analysed for significant difference by use of the Bartlett test (Appendix I). Similarly, Table 2.9.4. shows the strength of buffer to have no significant effect on SSA hydrolysis when compared by Student 't' test.

pH	Buffer System	Strength	First Order Rate Constant $\pm$ SE (sec^{-1})	't' test *
2.20	Clark and Lubs KCl:HCl	single	$1.005 \pm 0.028 \times 10^{-6}$	t_{obs} =
		half	$1.023 \pm 0.048 \times 10^{-6}$	0.324
6.05	McIlvaines Citrate:Phosphate	single	$1.557 \pm 0.011 \times 10^{-5}$	t_{obs} =
		half	$1.709 \pm 0.089 \times 10^{-5}$	1.700
7.75	Gomoris tris:HCl	single	$3.688 \pm 0.066 \times 10^{-5}$	t_{obs} =
		half	$3.613 \pm 0.036 \times 10^{-5}$	0.998
8.35	Sorensens glycine:NaOH	single	$8.482 \pm 0.026 \times 10^{-5}$	t_{obs} =
		half	$8.517 \pm 0.037 \times 10^{-5}$	0.772

* In all cases $t_{tab} = 2.306$, $N = 12$, $p' = 0.05$

Table 2.9.4. Effect of Buffer Strength on the Apparent First Order Rate Constant for SSA Hydrolysis at 50°C.

pH	Buffer System	First Order Rate Constant ± SE (sec^{-1})	Bartlett χ^2 value
2.20	Clark and Lubs KCl:HCl	$1.005 \pm 0.028 \times 10^{-6}$ $1.050 \pm 0.037 \times 10^{-6}$	27.12
	McIlvaines Citrate:Phosphate	$1.161 \pm 0.031 \times 10^{-6}$ $9.643 \pm 0.391 \times 10^{-7}$	$\chi^2_{tab} = 35.71$ N = 24 p′ = 0.05
6.95	McIlvaines Citrate:Phosphate	$1.904 \pm 0.039 \times 10^{-5}$ $1.877 \pm 0.033 \times 10^{-5}$	0.017
	Gomoris tris HCl	$1.869 \pm 0.043 \times 10^{-5}$ $1.880 \pm 0.043 \times 10^{-5}$	$\chi^2_{tab} = 35.71$ N = 24 p′ = 0.05
7.75	Gomoris tris HCl	$3.688 \pm 0.066 \times 10^{-5}$ $3.465 \pm 0.156 \times 10^{-5}$	1.004
	Sørensens Glycine:NaOH	$3.446 \pm 0.066 \times 10^{-5}$ $3.494 \pm 0.064 \times 10^{-5}$	$\chi^2_{tab} = 35.71$ N = 24 p′ = 0.05

Table 2.9.3 Effect of Buffer Type on the Apparent First Order Rate Constant for SSA Hydrolysis at 50°C.

2.9.4 INFLUENCE OF pH

The stability of SSA was studied as a function of pH at 50° utilising the buffer systems and methodology previously described (See 2.9.2), typical plots being shown in Fig. 2.9.2. The apparent first order rate constants calculated from such log % residual concentration vs time plots for pH 0.65 - 13.0 are shown in Fig. 2.9.3 vs pH. Since SSA is capable of ionising into one non-ionised and two ionic species, equation (14) can be rewritten to incorporate terms describing the contribution of these species to the overall rate constant, thus:-

$$-\frac{dc}{dt} = \left[SSA\right]\left[k_o + k_1\left[H_3O^+\right] + k_3\left[OH^-\right]\right] + \left[SSA^-\right]\left[k_4 + k_5\left[H_3O^+\right] + k_6\left[OH^-\right]\right] + \left[SSA^=\right]\left[k_7 + k_8\left[H_3O^+\right] + k_9\left[OH^-\right]\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (28)$$

where $\left[SSA\right]$, $\left[SSA^-\right]$ and $\left[SSA^=\right]$ are the concentrations of the non-ionised, mono- and di-ionised species of SSA respectively. The different susceptabilities of the three ionic forms of SSA to hydronium ion-, hydroxyl ion- and water-catalysed hydrolysis is probably responsible for the plateaus and inflections of the pH-stability profile shown in Fig. 2.9.3.

Specific hydronium and hydroxyl ion-catalysed hydrolysis should, according to equations (12) and (13), be represented by linear plots of the observed first order hydrolysis rate constant vs pH with negative and positive slopes respectively of 1. Examination of Fig. 2.4.3 shows that although sections (A - B; B - C and D - E) of the pH-stability profile can be represented by straight lines, the slope of those lines are not 1, the values, calculated by linear least-squares regression analysis being

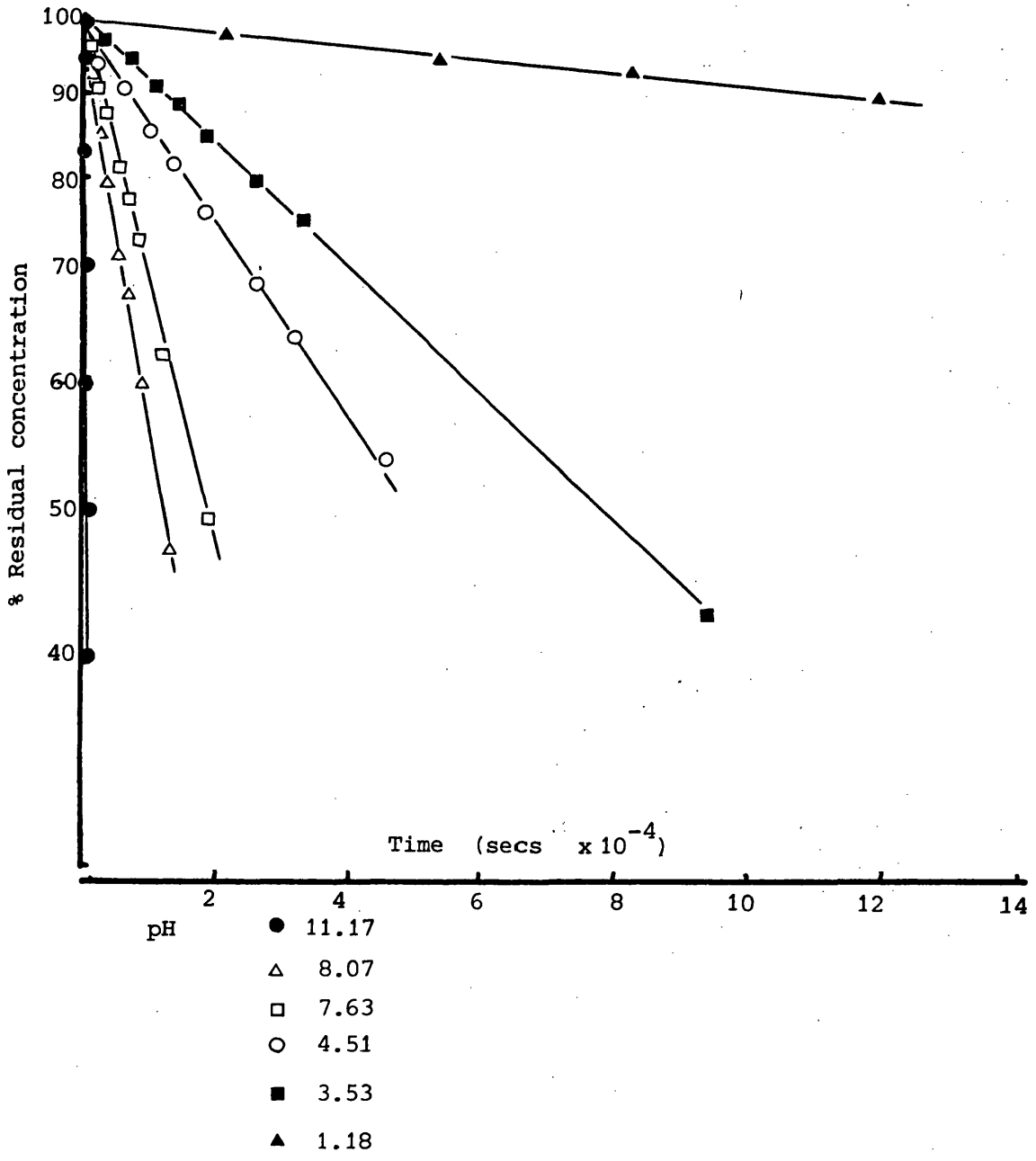


Fig. 2.9.2 Influence Of pH On SSA Hydrolysis At 50°.

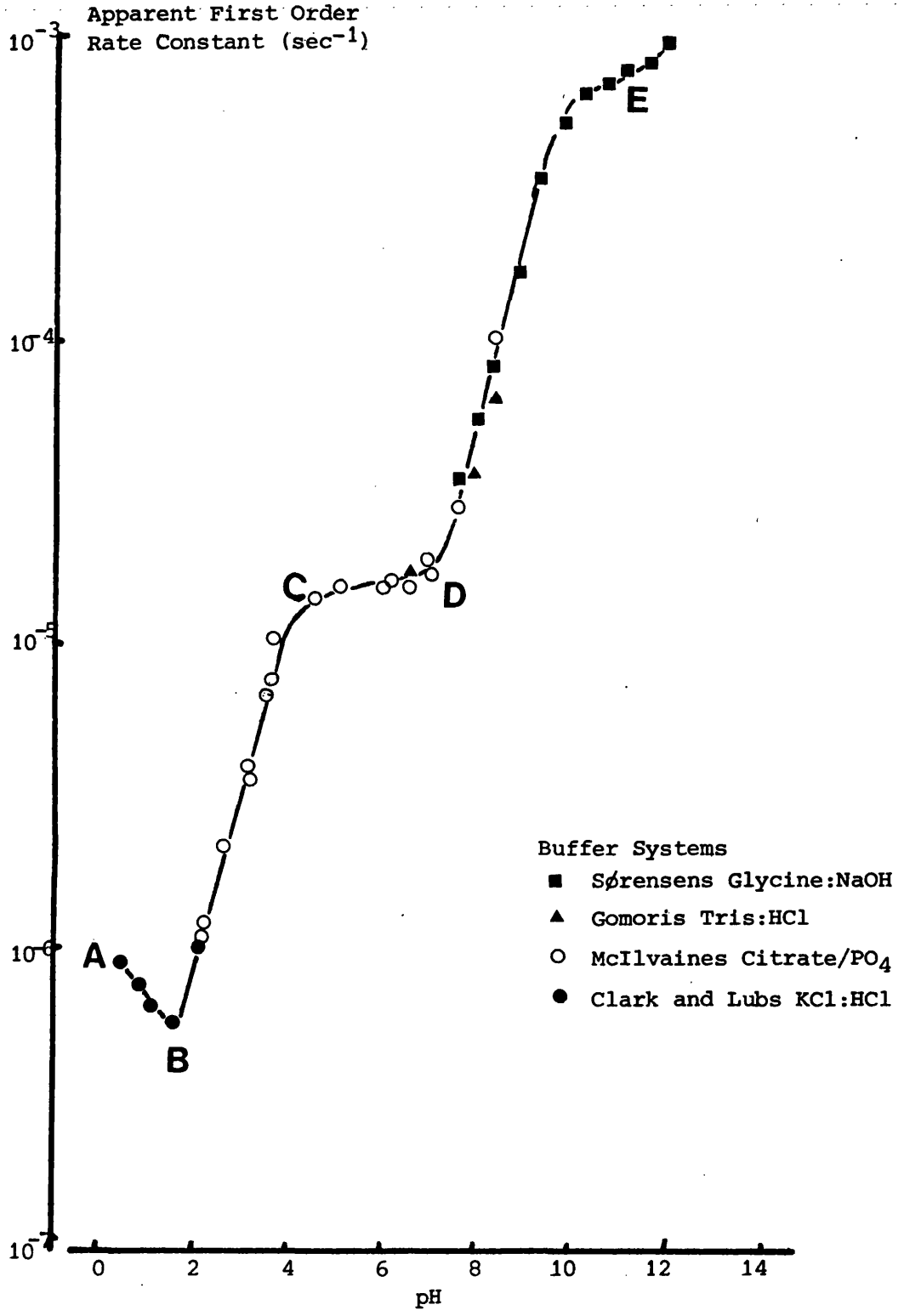


Fig. 2.9.3 pH Profile For The Hydrolysis Of SSA At 50°

- 0.244, + 0.635 and + 0.592 respectively. Other factors must therefore be operating over these pH ranges that determine the measured rates of reaction, but further investigations on this were not carried out.

2.9.5 INFLUENCE OF TEMPERATURE

The effect of temperature on the stability of SSA was studied at pH 3.12, 5.15 and 8.15 by the method previously described (See 2.9.2), the pH of each solution being adjusted, at the appropriate temperature, to the required value by the addition of N HCl or NaOH where necessary. pH 3.12 and 8.15 were chosen since the pH stability profile (Fig. 2.9.3) showed hydroxyl-ion catalysis for the hydrolysis of the mono- and di-ionised species of SSA respectively, and pH 5.15 represented a stage of transition between the two.

The influence of temperature on reaction rate is given by the Arrhenius equation:-

$$k = Ae^{-E/RT}$$

$$\text{or} \quad \log k = \log A - \frac{E}{2.303R} \cdot \frac{1}{T} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (29)$$

where k is the specific reaction rate, A is a constant, the frequency factor, E is the activation energy, R is the gas constant ($8.3143\ J^{o}K^{-1}mole^{-1}$) and T the absolute temperature. It is evident from equation (29) that a plot of log k vs $^{1}/_{T}$ will have a slope of $-\ ^{E}/_{2.303R}$ and an intercept of log A.

The apparent first order rate constants obtained, shown in Table 2.9.5, are graphically expressed in Fig. 2.9.4 according

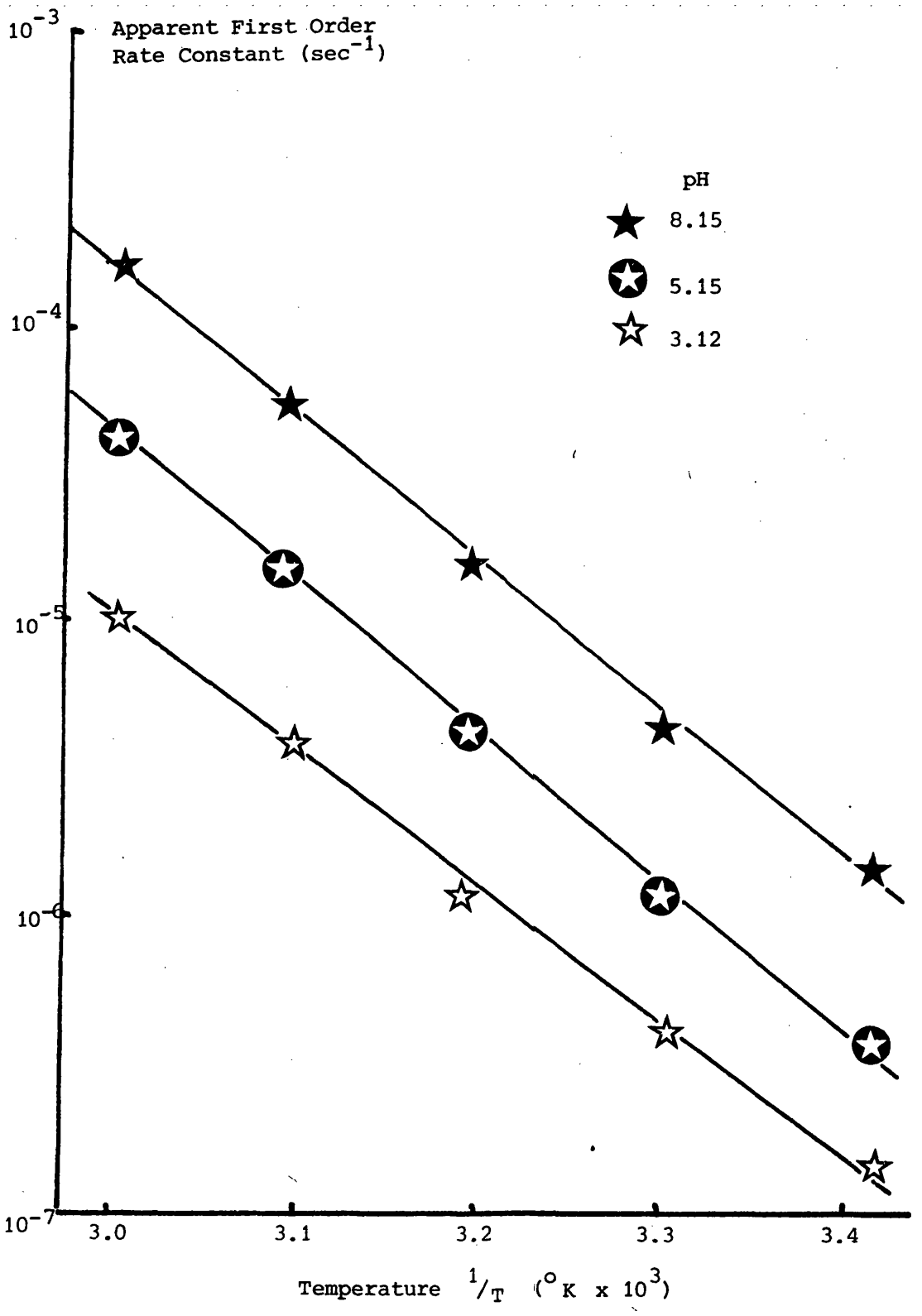


Fig. 2.9.4 Arrhenius Plots For SSA Hydrolysis At pH 3.12, 5.15 And 8.15.

to equation (29) and the data at each pH was submitted to linear least-squares regression analysis to provide values from which E and A were calculated.

Temperature		Apparent First Order Rate Constant (sec^{-1})		
^{o}C	$1/_{T}A^{o}$ ($x10^{-3}$)	pH 3.12	pH 5.15	pH 8.15
19.85	3.415	1.45×10^{-7}	3.48×10^{-7}	1.45×10^{-6}
29.60	3.305	4.05×10^{-7}	1.18×10^{-6}	4.21×10^{-6}
40.05	3.194	1.15×10^{-6}	4.23×10^{-6}	1.60×10^{-5}
50.00	3.096	3.84×10^{-6}	1.51×10^{-5}	5.40×10^{-5}
59.95	3.003	1.02×10^{-5}	4.36×10^{-5}	1.47×10^{-4}

Table 2.9.5 Influence of Temperature on SSA Hydrolysis at pH 3.12, 5.15 and 8.15

Activation energies for the influence of temperature on SSA hydrolysis at pH 3.12, 5.15 and 8.15 were calculated to be 86.42 ± 2.68, 98.22 ± 1.47 and 94.83 ± 2.24 $KJ^{o}K^{-1}mole^{-1}$ respectively. These values are within the range expected for ester hydrolysis. Departure from linearity for Arrhenius plots usually is indicative of a complex mechanism, or a change in the dominant mechanism, of degradation over the range studied. The linearity of the plots given in Fig. 2.9.4 suggests therefore that the overall mechanism of the hydrolytic degradation of SSA does not change with temperature. The differences in activation energies might reflect changes in the ratios of the ionic species undergoing hydrolysis since the pK_a of SSA can be expected to

change with temperature also, thereby altering the proportions of the ionised to non-ionised species. Where the ionic nature of the molecule undergoing hydrolysis changes, then this will modify the electrophi lic nature of the molecule. An ionic species with a high affinity for the catalytic hydroxyl ion would be expected to reflect itself in a significantly lower activation energy, so that the value at pH 3.12 would be expected to be lower than that at pH 8.15 as is found, the values being 86.42 ± 2.68 and 94.83 ± 2.24 $KJ^{o}K^{-1}mole^{-1}$ respectively. Where the ionic nature of the molecule remains unchanged and the nature of the preponderant catalytic species, ie H_3O^+ or OH^-, varies then a more complex picture results where both the activation energy and the frequency factor would be expected to change. This may provide an explanation for the non-parallelism of the Arrhenius plots shown and the frequency factors of 3.469×10^8, 1.097×10^{11} and 1.097×10^{11} for pH 3.12, 5.15 and 8.15 respectively.

2.10 RECRYSTALLISATION OF SSA FROM DIFFERENT MEDIA

The anticipated oral administration of SSA to man, in the form of a solution of the sodium salt, required that the influence of gastric juice on its in vivo precipitation should be investigated. Precipitation can be expected since SSA is poorly soluble under acidic conditions (80 $\mu g.ml^{-1}$ at 37^{o} in 0.1N HCl). If it is assumed that the volume of human gastric fluid is approximately 100 ml, and of pH 1.0, then these conditions may be simulated in vitro to provide a model for the examination of the influence of gastric juice and other experimental media on the precipitation of SSA from solution.

Method. The experimental design was essentially the same as that used for the solubility studies previously reported (See 2.6.1) except that the agitation intensity was controlled at 52 $rev.min^{-1}$ by use of a constant speed motor (Parvalux, Bournemouth) fitted with a flat-bladed stirring paddle. The apparatus is schematically shown in Fig. 2.10.1.

Approximately 60 mg SSA was accurately weighed and dissolved in 1 ml 2N NaOH. This solution was rapidly diluted with 200 ml of 0.0015N HCl and immediately added to 100 ml of the test solvent system; both solutions were previously equilibriated at 37^{o}. 2 ml samples were removed by millipore filtration at t = 0 and at appropriate time intervals and assayed for SSA as described (See 2.5). The pH was monitored throughout the time course of each experiment and was found not to change in any of the systems. Samples of material precipitated after 24 hours were removed by filtration through sintered glass and dried under vacuum overnight prior to photomicrographic and X-ray diffraction examination for differences in habit and morphology respectively.

The solvent systems used were:-

a) 0.1N HCl, as a reference.

b) Simulated gastric juice (U.S.P. 1975), adjusted to pH 1.0 with N HCl.

c) Half-diluted human gastric juice obtained by intubation from patients in a pre-operative situation. Samples from one subject, taken on one occasion, were pooled and diluted with an equal volume of water to provide sufficient volume for duplicate studies.

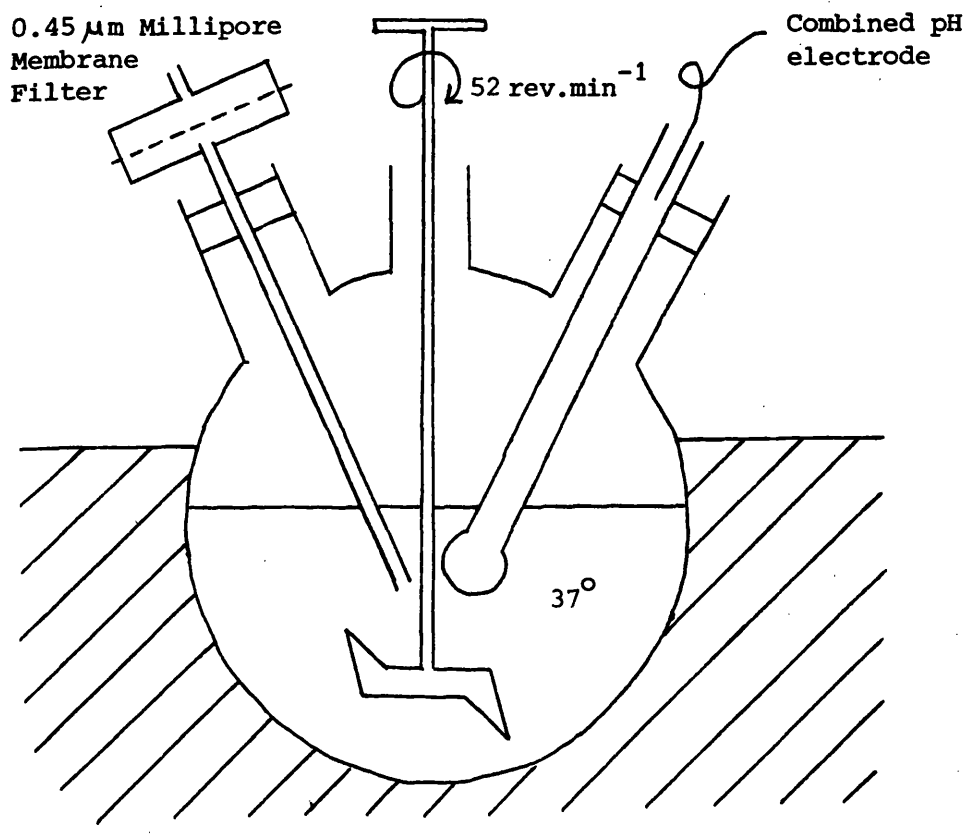


Fig. 2.10.1 Recrystallisation Apparatus

d) Polyvinylpyrrolidone PVP (mol.wt. 44,000) of 0.1% and 2% w/v in 0.1N HCl.

Plots for the concentration of SSA remaining in solution vs time are given in Fig. 2.10.2 for each of the systems studied. It is evident that simulated and natural gastric juices and both PVP solutions considerably retarded the precipitation of SSA when compared to that shown for 0.1N HCl alone. The initial fall in SSA concentration in both gastric juice systems was observed on each of three occasions this study was performed but was not present for either of the PVP solutions.

Photomicrographic analysis of precipitated material was achieved under the light microscope (Beck Kassel) fitted with a single lens reflex camera body (Praktica Super T.L.). Representative photomicrographs shown in Fig. 2.10.3 clearly demonstrate the gross differences in size, number and shape of the material precipitated from the various solvent systems. All the photomicrographs shown are of the same magnification and have been identically treated during printing.

X-ray diffraction patterns for the precipitates were obtained by use of the powder film camera technique previously described (See 2.2.1). This method of examination had to be used since the amount of precipitate produced in some of the solvent systems (PVP) was very small. Resultant densitometer tracings are given in Fig. 2.10.4 with SSA, as supplied, for reference. The coincidence of the diffraction peaks verifies that, although the precipitates differ greatly in size and habit, they exhibit crystalline patterns that are identical with that for the parent

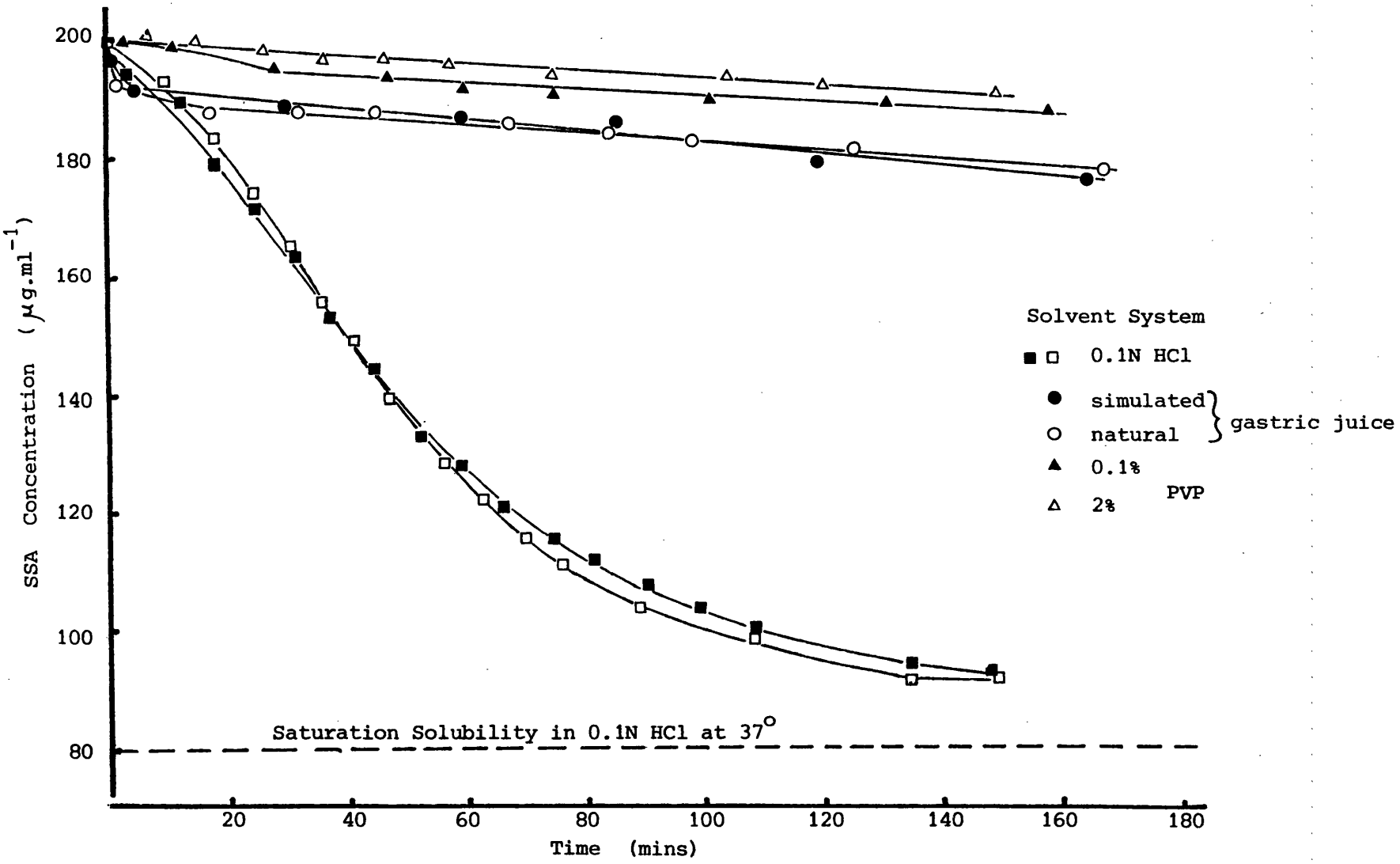


Fig. 2.10.2 Amount Of SSA Remaining In Solution After Addition Of SSA (60 mg In Solution) To 0.1N HCl, Simulated And Natural Gastric Juice And PVP Solutions (0.1% and 2%).

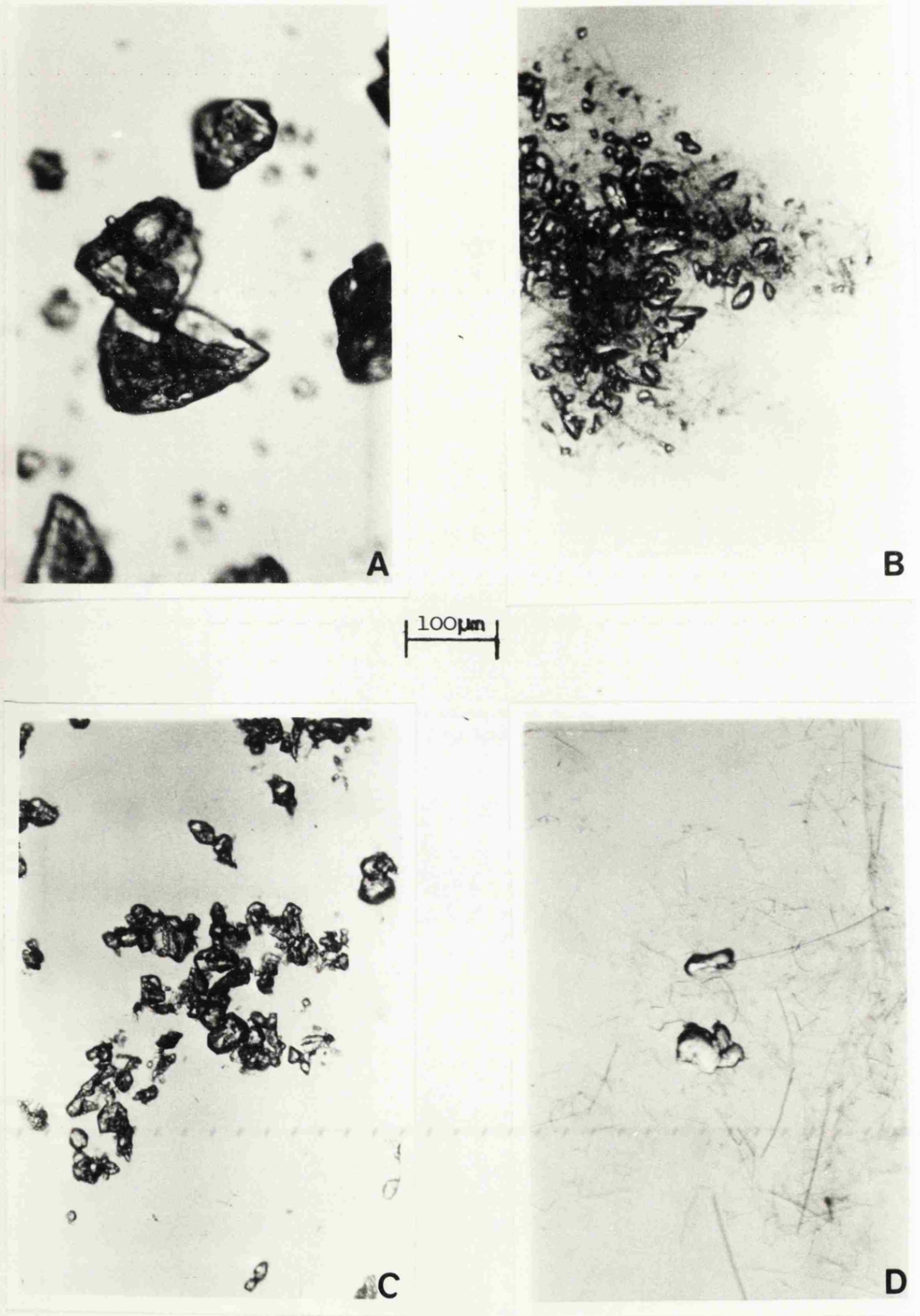


Fig. 2.10.3 Photomicrographs of SSA Recrystallized from 0.1N HCl(A) Simulated Gastric Juice (B), Human Gastric Juice (C) and 2% PVP (D) at pH 1.0 and 37°.

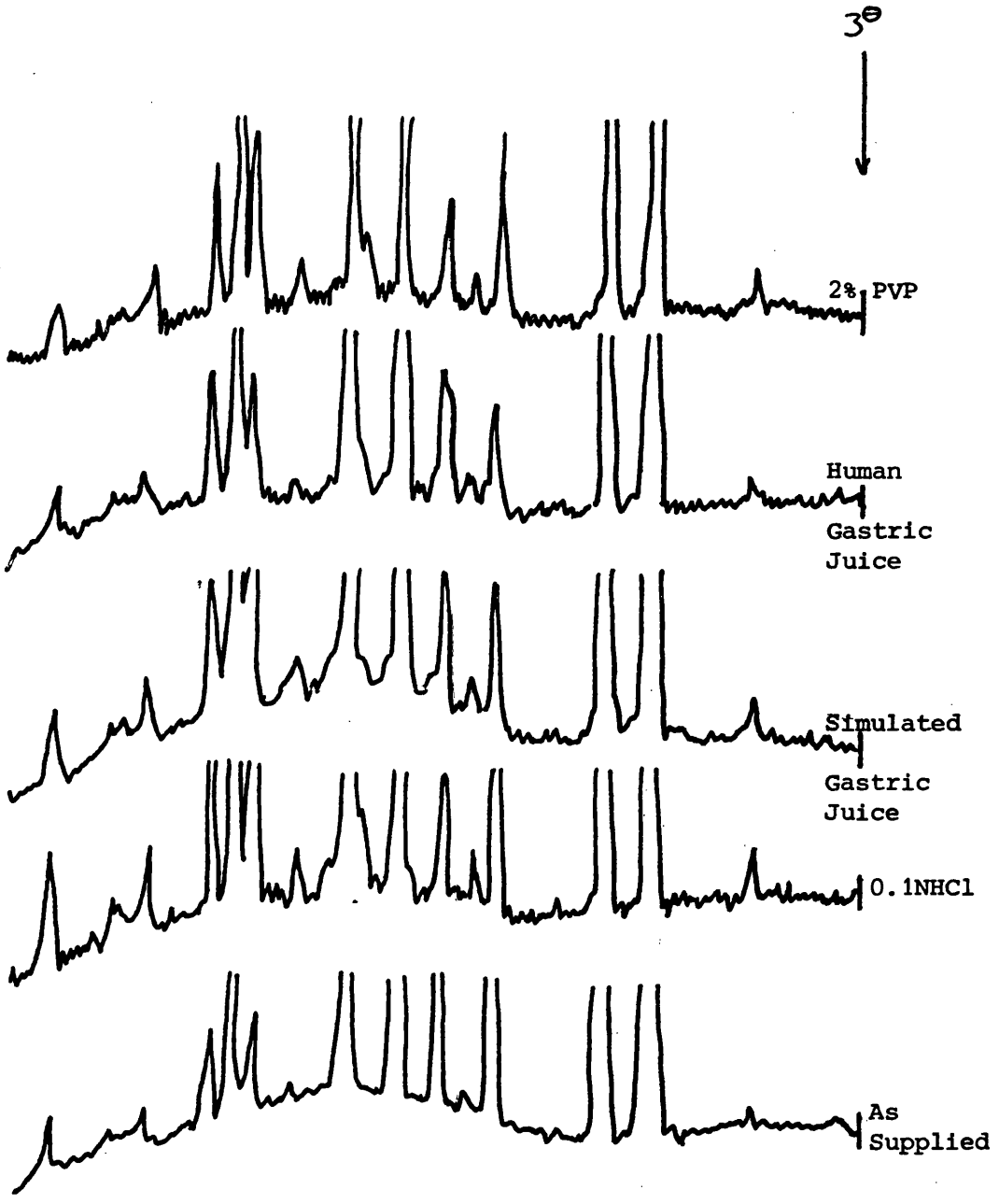


Fig. 2.10.4 X-Ray Diffraction Patterns For SSA Recrystallised From 0.1N Hydrochloric Acid, Simulated and Natural Gastric Juice and 2% PVP At pH 1.0 and 37°.

material. It may be concluded, therefore, that PVP and some constituent of both gastric juice systems, while preventing and/or delaying SSA recrystallisation, do not alter the morphological nature of the product.

The significance of these observations will be discussed later.

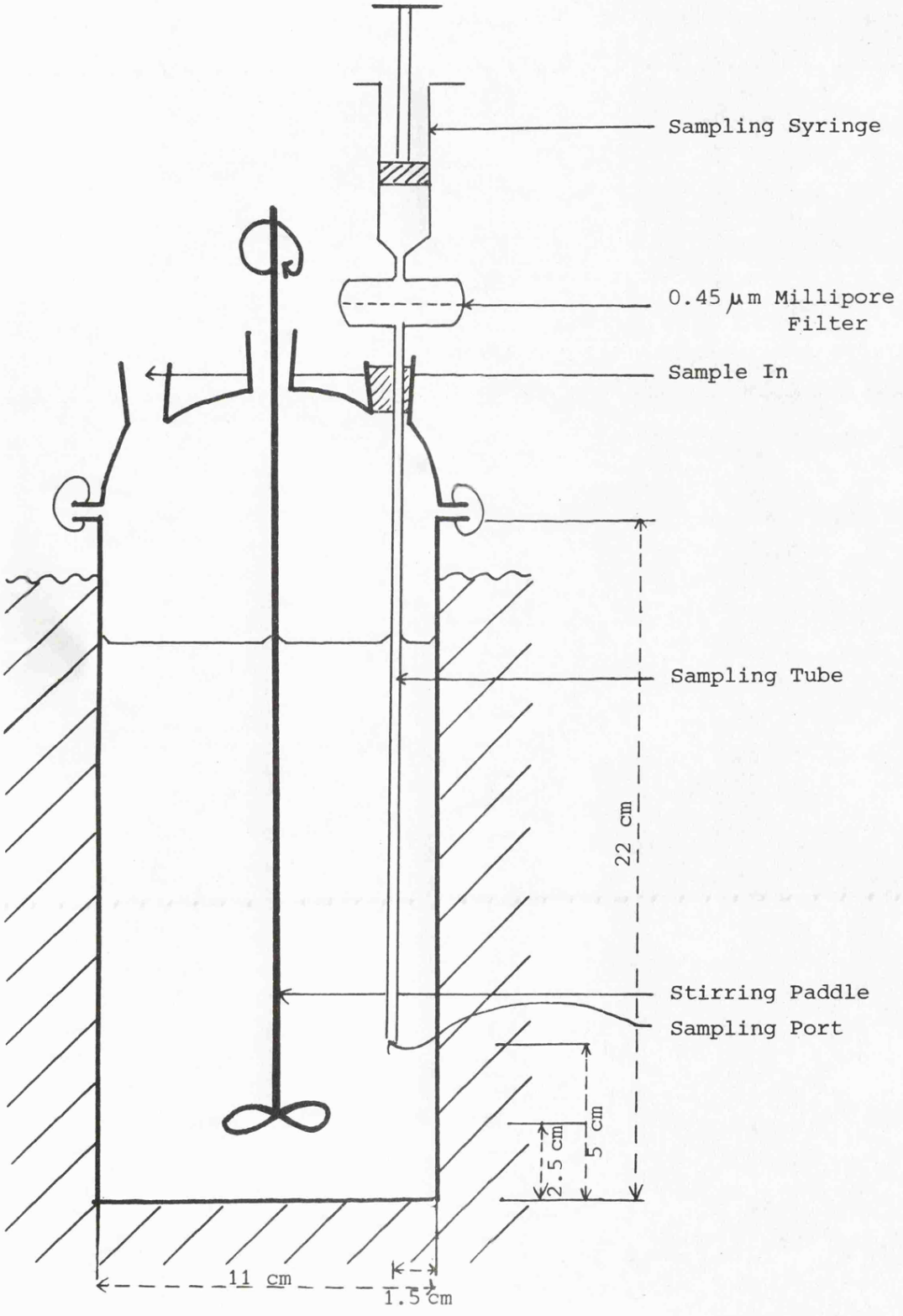
2.11 DETERMINATION OF THE DISSOLUTION RATE OF SSA IN 0.1N HCL AS A FUNCTION OF PARTICLE SIZE.

2.11.1 APPARATUS AND METHOD

The apparatus used (Fig. 2.11.1) was based on the stirred-beaker method of Levy and Hayes (1960). The vessel, containing 1500 ml of 0.1N HCl was partially immersed in a thermostatically-controlled waterbath at 37 $\pm$ 0.1^{o}C. Stirring was controlled by a constant speed motor at 52 rev.min^{-1} (Parvalux, Bournemouth). At the commencement of each experiment the stirring rate was checked by a stroboscope (Flash Tac. Electronic Applications, Hitchin). Removal and separation of samples from undissolved material was achieved by the millipore membrane system previously described (See 2.6). At each sampling time the 6 ml taken was replaced with an equal volume of solvent, introduced via the millipore filter. This ensured a constant volume and returned any undissolved particles from the surface of the membrane to the bulk solution. The apparatus was constructed such that the sampling assembly and the stirrer paddle were always at a fixed position in relation to the sides and bottom of the vessel.

Method. 200 mg of SSA was placed on the surface of the dissolution

Fig. 2.11.1 Apparatus For Powder Dissolution Studies



medium equilibriated at 37^{o} and at appropriate time intervals 6 ml samples were removed, from which 5 ml was accurately pipetted and assayed according to the UV method described (See 2.5) for SSA content.

2.11.2 TREATMENT OF DATA

The appearance of drug, from dissolving particles, into solution can be expressed in several ways, the most common of which is to plot the amount dissolved vs time to produce a dissolution-time profile. From such a plot a quantitative assessment of the rate can be made by measuring the time taken for a particular weight of material to dissolve, or alternatively, by measuring the amount dissolved after a specified time. These types of representation have been successfully employed in the correlation of in vitro dissolution with in vivo absorption for several drugs including aspirin (Levy, 1963; Wood, 1967), tetracycline (Macdonald et al, 1969) and salicylamide (Bates et al, 1969). For some compounds the rate of dissolution can be wholly described by first order kinetics and the apparent first order rate constants can therefore be used as a reference for comparison with other in vitro studies and/or with first order in vivo absorption processes for the same drug. Representation of dissolution data to yield apparent first order rate constants is based on the following rationale.

Under sink conditions, and where dissolution follows first order kinetics, it can be assumed that the surface area (S) decreases exponentially with time, ie,

$$S = S^{o} e^{-ks\ (t-to)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (30)$$

where S^o is the surface area available for dissolution at the time when the apparent first order dissolution phase commences at time, t_o, and ks is the rate constant that describes the change in surface area. Under sink conditions the concentration of the drug in solution, (C), at time t is very much less than the saturation solubility (C_s) ie $C \ll 10\% \ C_s$, and can therefore be ignored. Under these conditions the Noyes Whitney equation (2) reduces to:-

$$\frac{dc}{dt} = k\ (C_s) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (31)$$

The amount of drug in solution (W) can be obtained by incorporation of a term relating to the volume (V) of the dissolution medium into equation (31) to give:-

$$\frac{dW}{dt} = K' C_s \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (32)$$

In this case $K' = kV$. Incorporation of a term for S (equation (30)) into equation (32) yields:-

$$\frac{dW}{dt} = K' C_s S^o e^{-ks\ (t-t_o)} \text{ for } t \geqslant t_o \ldots\ldots\ldots \quad (33)$$

which integrates to give:-

$$W = W_o + \frac{K}{ks}\ C_s\ S^o \left[1-e^{-ks(t-to)}\right]$$

$$= W_o + W_m \left[1-e^{-ks(t-to)}\right] \quad \text{for } t \geqslant t_o \ldots\ldots \quad (34)$$

where W_m is related to the change in weight associated with the change in surface area ($W_m = (K/_{ks}) C_s S^o$) and W_o is the amount

dissolved at time, t_o. Therefore:-

$$W_\infty = W_o + W_m \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (35)$$

where W_∞ is the amount in solution at infinite time. Rearrangement of equation (34) and substitution of W_∞ for $W_o + W_m$ gives:-

$$W_\infty - W = W_m e^{-ks(t-to)} \quad \text{for } t \geqslant t_o \quad \ldots\ldots\ldots\ldots\ldots \quad (36)$$

Taking logarithms of both sides we get:-

$$\log (W_\infty - W) = \log W_m - \frac{ks}{2.303} (t-to) \quad \text{for } t \geqslant t_o \quad \ldots\ldots\ldots \quad (37)$$

where $W_\infty - W$ is the amount undissolved at time t and W_m is the amount finally dissolved. Examination of equation (37) shows that a plot of log $(W_\infty - W)$ vs time is linear, with an intercept of log W_m and a slope of $-\frac{ks}{2.303}$, if dissolution can be adequately described by first order kinetics. Deviations of such plots from linearity, in the initial and final stages of dissolution of a solid dosage form, can be attributed to alterations in surface area associated with disintegration, deaggregation and complete dissolution of the smaller particles respectively. Wagner (1969) suggested a method for the determination of apparent first order dissolution rate constants for disintegrating tablets and capsules that was based on the change of surface area with time. His method accounted for the initial lag time before which first order kinetics applies, and for the final phase associated with small particle dissolution. Treatment of SSA dissolution data by the Wagner method was not

valid since surface area changes for SSA dissolution could be attributed to wetting in the first place and the ultimate settling of undissolved material to the bottom of the vessel. Apparent first order dissolution rate constants for SSA could however be calculated from a linear section of the log % undissolved vs time plot for each study.

The data for SSA dissolution studies can therefore be represented as both a dissolution profile and according to equation (37). From the latter apparent first order dissolution rate constants can be calculated.

2.11.3 PRELIMINARY OBSERVATIONS

Initial observations indicated that SSA was only poorly wetted when placed on the surface of the dissolution medium. The technique was therefore considered unlikely to give reproducible results and the influence of wetting agent was therefore investigated. The non-ionic surface active agent, polysorbate 80 (Honeywill-Atlas, Surrey) was chosen and preliminary studies showed that a concentration of 5×10^{-3}% w/v was sufficient to promote adequate wetting without significantly influencing the saturation solubility of the drug. Solubility studies carried out as previously described (See 2.6) gave a saturation solubility of SSA in 0.1N HCl containing 5×10^{-3}% w/v polysorbate 80 of $80.4 \pm 0.3\ \mu g.ml^{-1}$ at 37^{o}. Consequently 0.1N HCl containing 5×10^{-3}% w/v polysorbate 80 was used as the dissolution medium for all future studies.

Apparent first order dissolution rate constants for SSA of

180-250 μm particle size range were obtained using two similar sets of apparatus, comparison of which revealed that, even though the rate constants determined using either vessel were reproducible, there was a significant difference between vessels. This suggested that slight differences in vessel geometry would influence the dissolution process by changing the hydrodynamic characteristics of the vessel. In order to ensure consistency the same set of apparatus was therefore used on all occasions, and, in triplicate determinations of the apparent first order dissolution rate constant for 180-250 μm material, determined in the one apparatus, the values obtained were $1.101 \pm 0.069 \times 10^{-5}$ sec^{-1} respectively. These values are not significantly different when compared by the Student 't' test, and illustrate the reproducibility of the system.

2.11.4 INFLUENCE OF PARTICLE SIZE ON SSA DISSOLUTION

The dissolution of different particle size ranges of SSA was investigated, the dissolution vs time profiles of which are shown in Fig. 2.11.2. Apparent first order dissolution rate constants are given in Table 2.11.1. The relationships between BET surface area, particle size and dissolution is clearly evident from examination of Figs. 2.11.3 and 2.11.4, which show that, with the exception of the micronised material, there is a good correlation between dissolution rate and BET surface area ($r = 0.984$). The studies on micronised material show an anomalous result in that, although possessing the largest surface area ($0.7683\ m^{-2}g^{-1}$) its dissolution rate is much slower than expected. This is probably because aggregates of incompletely, wetted powder were formed even in the presence of polysorbate 80

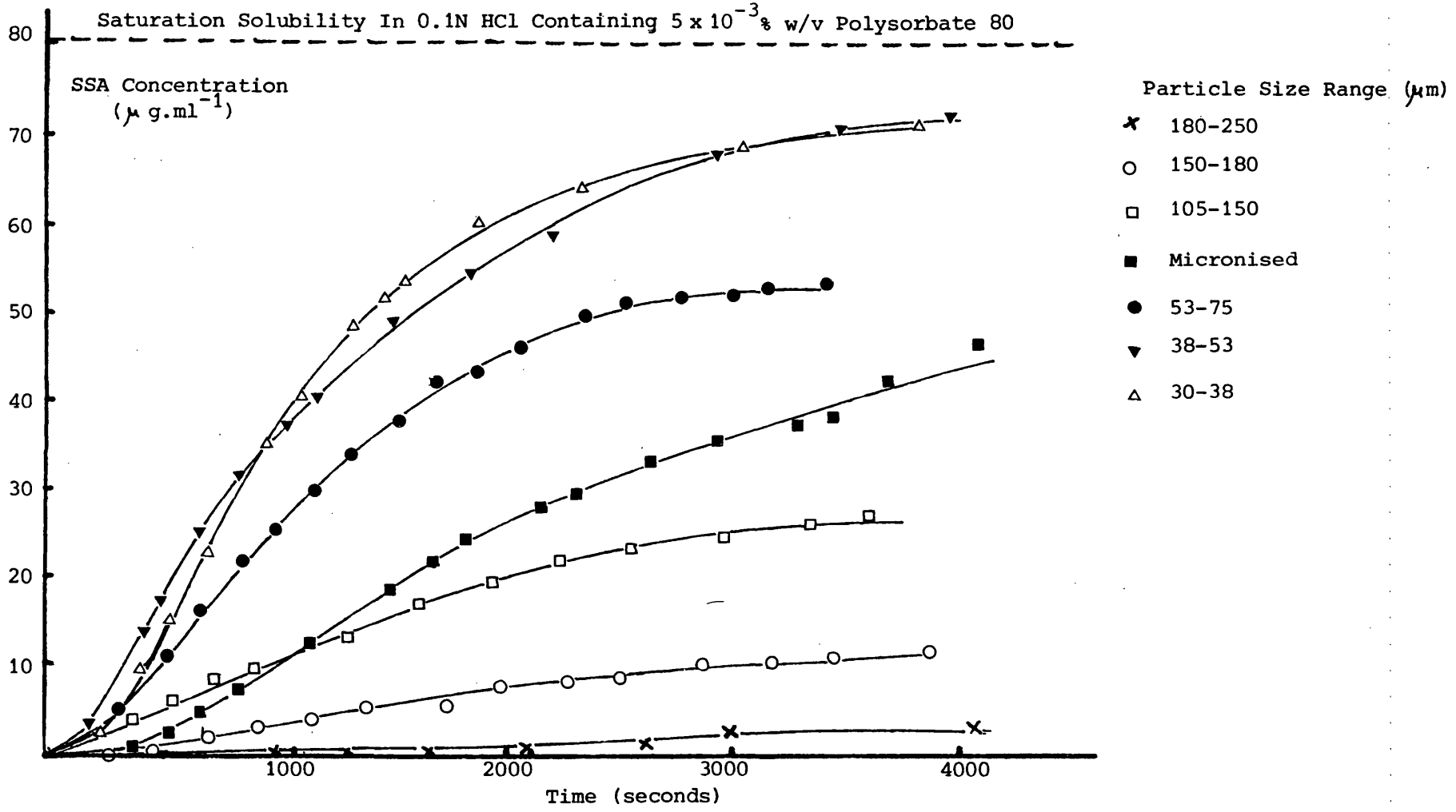


Fig. 2.11.2 Dissolution Profiles For SSA In 0.1N HCl Containing 5×10^{-3}% w/v Polysorbate 80 at 37°.

Particle Size Range (μm)	Particle Size Mean* (μm)	BET Surface Area ($m^2 g^{-1}$)	Apparent First Order Dissolution Rate Constant ± SE (x $10^{-4} sec^{-1}$)
180-250	215	0.070♣	0.118 ± 0.006
150-180	165	0.1105	0.399 ± 0.015
105-150	139	0.1156	1.350 ± 0.034
75-105	91.7	0.2892	3.707 ± 0.085
53-75	66.9	0.3606	4.132 ± 0.052
38-53	45.5	0.5025	6.432 ± 0.133
30-38	34	0.5111	7.910 ± 0.105
Micronised	5	0.7683	2.319 ± 0.041

* The mean particle size is calculated from a knowledge of the arithmetic mean of the sieve fractions previously bulked (See 2.4.1)

♣ Estimated by interpolation from Fig. 2.4.4.

Table 2.11.2 Relationship Between Particle Size, BET Surface Area and Apparent First Order Dissolution Rate Constant for SSA.

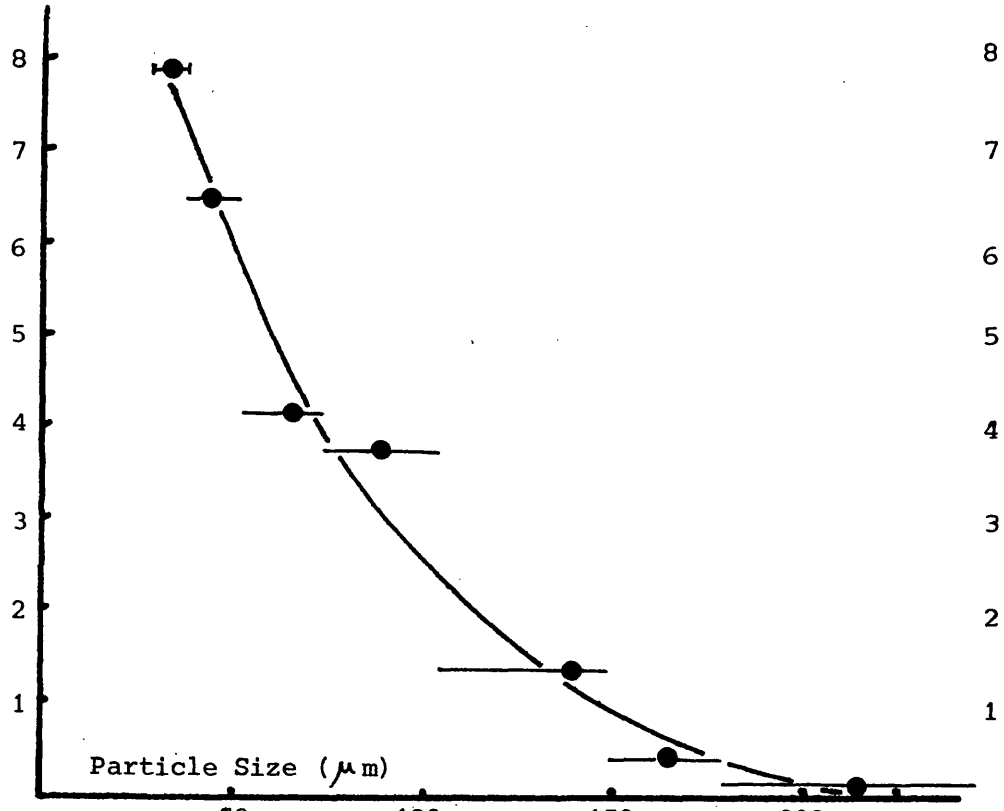


Fig. 2.11.3

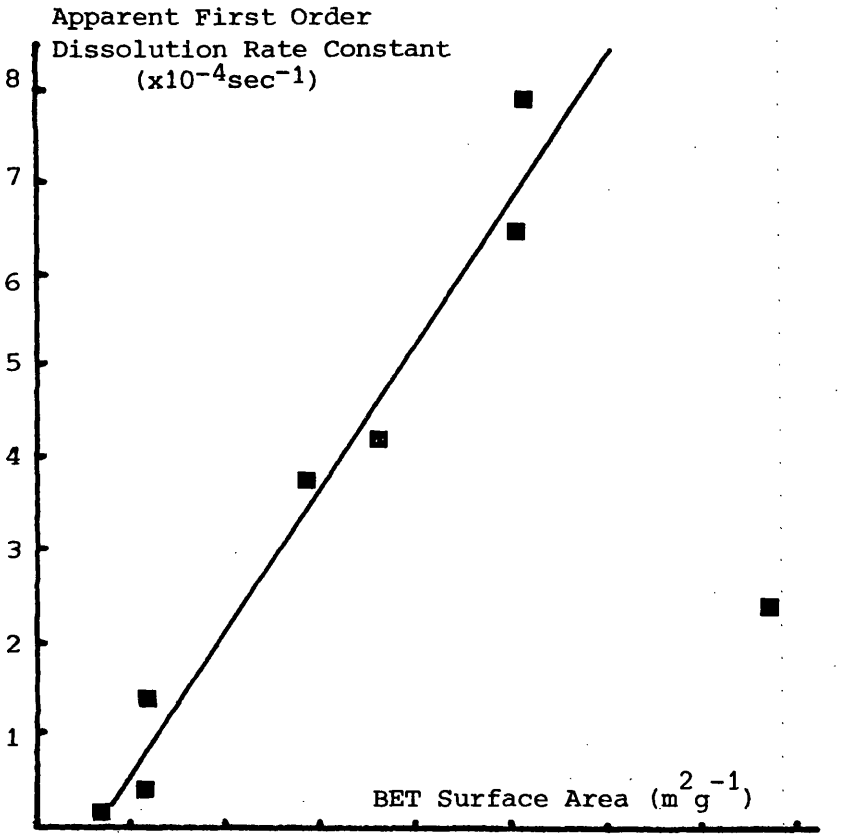


Fig. 2.11.4

Relationship Between Apparent First Order Dissolution Rate Constant And Particle Size (Fig. 2.11.3) And BET Surface Area (Fig. 2.11.4).

and the low dissolution rate is probably a reflection of a reduced effective surface area, a phenomenom that is not uncommon for microcrystalline powders that have a high electrostatic charge.

2.12 PREPARATION OF SSA COPRECIPITATES AND ADMIXTURES WITH POLYVINYL-PYRROLIDONE (PVP), CHOLIC ACID (CA) AND DEOXYCHOLIC ACID (DOCA).

2.12.1 SSA:PVP COPRECIPITATES

Coprecipitates of SSA:PVP in weight-to-weight ratios of 2:1, 1:1, 1:2, 1:5 and 1:10 were prepared by dissolving the appropriate amount of each in a common organic solvent, chloroform, the solvent was removed under vacuum, without heating above 40^{o}, in a rotary evaporator (Rotavapor-R, Buchi, Switzerland). However, not all of the solvent could be removed this way and a glass-like gum was produced for each SSA:PVP ratio. The gum formation was attributed to polymer plasticisation by chloroform. The final traces of solvent were removed by physical manipulation of the gum, with a metal spatula, under petroleum ether ($60-80^{o}$ fraction); this promoted precipitation of a pale yellow/white solid. With the 1:5 and 1:10 coprecipitates a large degree of physical effort was required to induce precipitation from the 'plasticised' gums which could only be achieved by grinding them, under petroleum ether, in a mortar. Final traces of solvent were removed by drying the coprecipitate to constant weight in a vacuum oven at 30^{o}. After drying, each sample was sieved to obtain the 75-105μm particle size fraction for subsequent in vivo studies and stored in glass jars in the desiccator.

2.12.2 SSA:CHOLIC ACID AND SSA:DEOXYCHOLIC ACID COPRECIPITATES

Cholic acid, CA, (mol.wt. 408.6) and deoxycholic acid, DOCA, (mol.wt. 392.6) were used as supplied (B.D.H.). Since the molecular weight of the bile acids was known the SSA:CA and SSA:DOCA coprecipitates (and admixtures) were prepared in molar ratios of 2:1, 1:1, 1:2, 1:5 and 1:10. The common solvent used for SSA:bile acid coprecipitate preparation was methanol since the bile acids were found to be insufficiently soluble in chloroform. The general method was the same as for SSA:PVP coprecipitates except that gums were not formed and the coprecipitates readily precipitated on solvent evaporation. A particle size fraction of 75-105 μm was again obtained by sieving and stored in glass jars in the desiccator for future use.

2.12.3 SSA:PVP, CA AND DOCA ADMIXTURES

These were prepared in the same ratios as their respective coprecipitates by physically mixing the appropriate amounts of each constituent in a closed jar. To enable direct comparisons between in vivo absorption data obtained from administration of the coprecipitate and admixture systems the latter were prepared from SSA, CA, DOCA and PVP of 75-105 μm particle size.

2.13 CHARACTERISATION OF SSA COPRECIPITATES

Material outside the 75-105 μm particle size range was retained to establish the SSA content of each ratio and for their X-ray diffraction analysis.

2.13.1 DETERMINATION OF SSA CONTENT

2.13.1.1 SSA:PVP Systems

Weighed amounts of each ratio, equivalent to approximately 50 mg SSA, were dissolved in 1 litre of water and assayed for SSA by ultraviolet spectrophotometry as described previously (See 2.5). The influence of PVP on the UV absorption of SSA at 341 nm in alkaline medium was investigated and found not to be significant for the concentrations used. The results of duplicate determinations are given in Table 2.13.1.

The total acid content of each SSA:PVP: coprecipitate was also determined by titration of a weighed sample (approx 220 mg SSA) with 0.1N NaOH as previously described (See 2.3.3). Blank titrations were performed on the appropriate, theoretically expected, amounts of PVP, the titres of which were subtracted from their respective SSA:PVP titre. The values for the PVP titres and the calculated SSA content of each coprecipitate are given in Tables 2.13.2 and 2.13.3 respectively.

		Weight PVP (mg)				
		100	200	400	1000	2000
Titre Volume 0.1N NaOH (ml)	a	0.12	0.21	0.42	1.10	2.20
	b	0.12	0.22	0.41	1.10	2.20
	c	0.12	0.21	0.43	1.15	2.22
	d	0.12	0.22	0.41	1.05	2.22
Mean		0.12	0.215	0.42	1.10	2.21

Table 2.13.2 Titres For PVP Against 0.1N NaOH.

SSA:PVP Ratio w:w	wt.SSA:PVP taken (mg)	Absorbance at 341 nm	wt.SSA Determined (mg)	% SSA
0	50.1	0.630	50.4	100.6
	50.3	0.633	50.6	100.7
			Mean	100.65
2:1	74.7	0.610	48.8	65.33
	74.9	0.640	51.2	64.89
			Mean	65.11
1:1	102.3	0.625	50.0	48.88
	97.8	0.610	48.8	49.90
			Mean	48.39
1:2	152.6	0.530	42.4	27.79
	160.4	0.545	43.6	27.18
			Mean	27.49
1:5	305.3	0.585	46.8	15.33
	301.6	0.575	46.0	15.25
			Mean	15.29
1:10	463.0	0.520	41.6	8.99
	469.0	0.530	42.4	9.04
			Mean	9.02

Table 2.13.1 Percentage Purity of SSA:PVP Coprecipitates as Determined by Ultraviolet Spectrophotometry

Coprecipitate SSA:PVP Ratio wt:wt	wt SSA:PVP Coprecipitate Taken (g)	Titre 0.1N NaOH (ml)	Corrected Titre (ml) *	% SSA
2:1	0.3064	7.82	7.70	64.86
	0.2987	7.77	7.65	66.10
	0.3080	7.92	7.80	65.36
	0.3082	7.98	7.86	65.82
			Mean	65.54
1:1	0.3975	7.66	7.44	48.30
	0.4083	7.98	7.76	49.05
	0.4133	8.07	7.85	49.02
	0.4132	7.97	7.75	48.41
			Mean	48.70
1:2	0.6168	7.57	7.15	29.92
	0.5945	7.34	6.92	30.04
	0.7268	8.94	8.52	30.25
	0.5839	7.17	6.75	29.84
			Mean	30.01
1:5	1.2056	8.48	7.38	15.80
	1.2201	8.50	7.40	15.65
	1.2095	8.41	7.31	15.60
	1.2015	8.33	7.23	15.53
			Mean	15.65
1:10	2.2132	9.90	7.69	8.97
	2.2323	9.92	7.71	8.91
	2.2128	9.80	7.59	8.85
	2.2025	9.88	7.67	8.99
			Mean	8.93

* Blank values are those obtained from Table 2.13.2.

Table 2.13.3 Percentage SSA Content of SSA:PVP Coprecipitates as Determined by Titration

A comparison of the experimentally determined % SSA purity with the theoretical value for each SSA:PVP coprecipitate is given in Table 2.13.4.

SSA:PVP Ratio	Mean % SSA Content		
	Theor.	UV Assay	Titration
2:1	66.67	65.11	65.54
1:1	50.00	49.39	48.70
1:2	33.33	27.49	30.10
1:5	16.67	15.29	15.65
1:10	9.09	9.02	8.93

Table 2.13.4 Comparison of Experimentally Determined % SSA Content of SSA:PVP Coprecipitates.

2.13.1.2 SSA:CA and SSA:DOCA Systems

The poor aqueous solubility of the bile acids precluded the use of the ultraviolet assay employed for the SSA:PVP systems. Assays were performed using ethanolic solutions of the coprecipitates. Nine standard solutions of SSA (approx. 1.8 to 118 $\mu g.ml^{-1}$, accurately known) were prepared in absolute ethanol. 5 ml volumes of each standard and 0.4N NaOH were adequately mixed and the absorbance read at the previously determined λ max of 345 nm against an ethanol/NaOH blank. Even though aqueous dilution of ethanol resulted in a reduced combined volume similar treatment of each standard and test sample should be similarly affected and the volume change was therefore ignored. Fig. 2.13.1 shows a standard calibration plot that adheres to the Beer Lambert Law. Submission of the data for the SSA standards

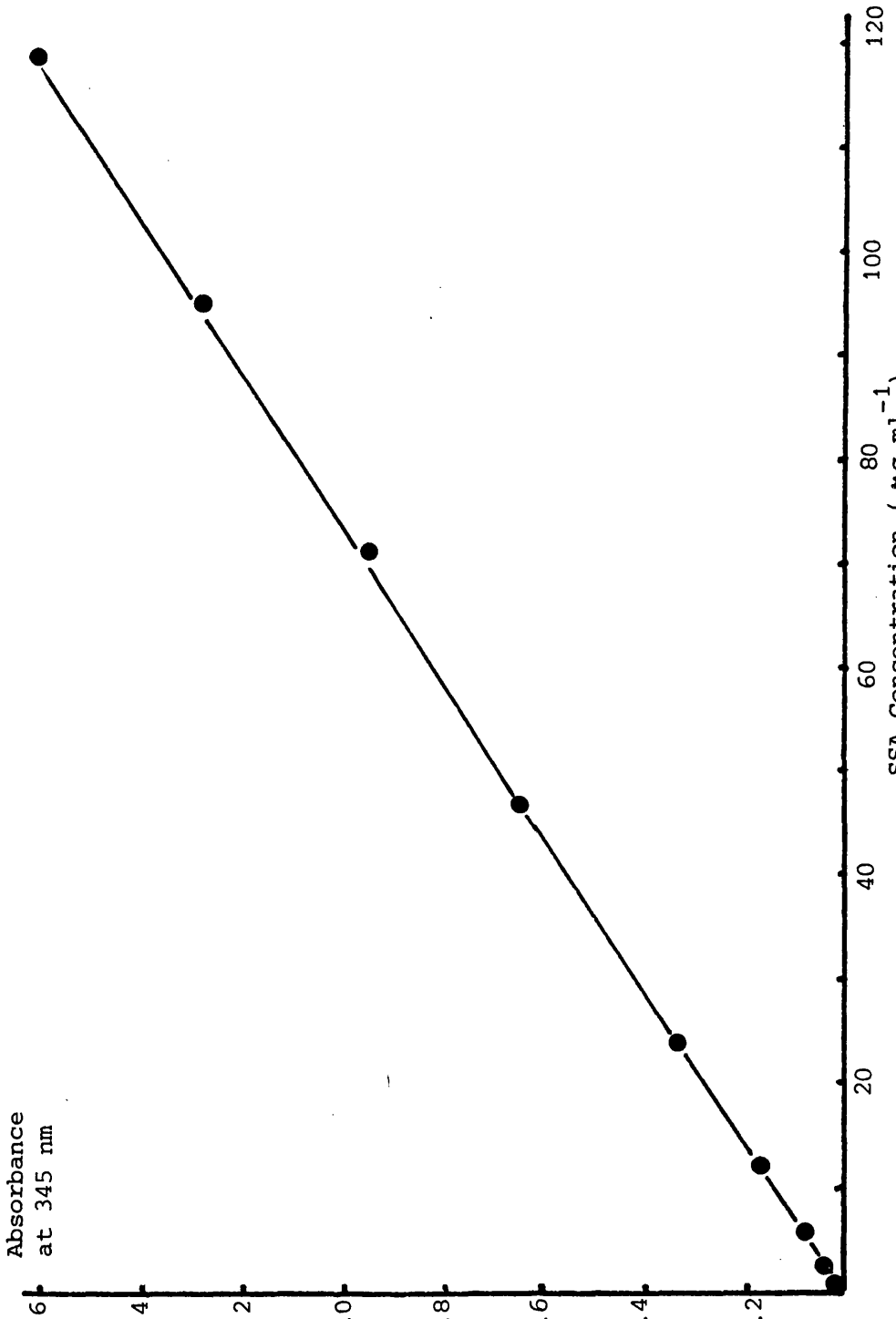


Fig. 2.13.1 Beer-Lambert Plot For SSA In Ethanol After Half Dilution With 0.4N NaOH

to linear least-squares regression analysis gave $E^{1\%}_{1cm}$ and ϵ values of 134.7 and 3476.1 respectively. Absorption spectra of identically-treated ethanol solutions of cholic and deoxycholic acids confirmed that neither absorbed at 345 nm nor induced changes in the absorbance spectrum for SSA under similar conditions.

Weighed amounts of each SSA:CA and SSA:DOCA coprecipitate ($\equiv$ approximately 50 mg SSA) were dissolved in 50 ml of absolute ethanol. 5 ml aliquots of each were assayed as described following a further 1 in 25 dilution with absolute ethanol. The results in Table 2.13.5 for SSA:CA and Table 2.13.6 for SSA:DOCA show good agreement with the theoretical % SSA content.

2.13.2 X-RAY DIFFRACTION EXAMINATION OF SSA:PVP COPRECIPITATES AND ADMIXTURES

Each of the SSA:PVP coprecipitates were examined by X-ray diffraction in order to establish whether or not the process of coprecipitation had altered the crystalline nature of the original constituents. The diffraction patterns were obtained using a Debye-Scherer film camera (See 2.2.1) and the resultant films used to give the densitometer tracings shown in Fig. 2.13.2. The tracings show that although SSA is highly crystalline, after its coprecipitation with PVP, in whatever ratio, the product showed no crystalline pattern and can be considered amorphous; PVP alone also showed amorphous behaviour.

Examination of the SSA:PVP admixtures by X-ray diffraction

SSA:CA Molar Ratio	wt taken (mg)	Absorbance at 345 nm	Dilution (x)	wt. SSA* determined (mg)	% SSA Content Experimental	% SSA Content Theoretical
2:1	74.3	0.433	25	40.18	54.08	55.82
		0.434	25	40.28	54.21	
				Mean	54.15	
1:1	90.8	0.388	25	36.01	39.66	38.71
		0.386	25	35.82	39.45	
				Mean	39.56	
1:2	140.5	0.370	25	34.36	24.46	24.00
		0.371	25	34.43	24.51	
				Mean	24.49	
1:5	292.3	0.362	25	33.59	11.49	11.22
		0.363	25	33.69	11.53	
				Mean	11.51	
1:10	529.6	0.310	25	28.77	5.43	5.94
		0.309	25	28.68	5.42	
				Mean	5.425	

* Corrected for 50 ml original volume.

Table 2.13.5 Percentage Purity of SSA:CA Coprecipitates Determined By UV Spectrophotometry

SSA: DOCA Ratio	wt taken (mg)	Absorbance at 345 nm	Dilution (x)	wt. SSA * determined (mg)	% SSA Content Experimental	% SSA Content Theoretical
2:1	92.3	0.568	25	52.71	57.19	56.79
1:1	124.7	0.532	25	49.37	39.59	39.66
1:2	200.1	0.520	25	48.26	24.12	24.74
1:5	437.1	0.566	25	52.53	12.02	11.62
1:10	826.9	0.478	25	44.36	5.36	5.17

* Corrected for 50 ml original volume.

Table 2.13.6 Percentage Purity of SSA:DOCA Coprecipitates Determined By UV Spectrophotometry. (Single Determinations Only).

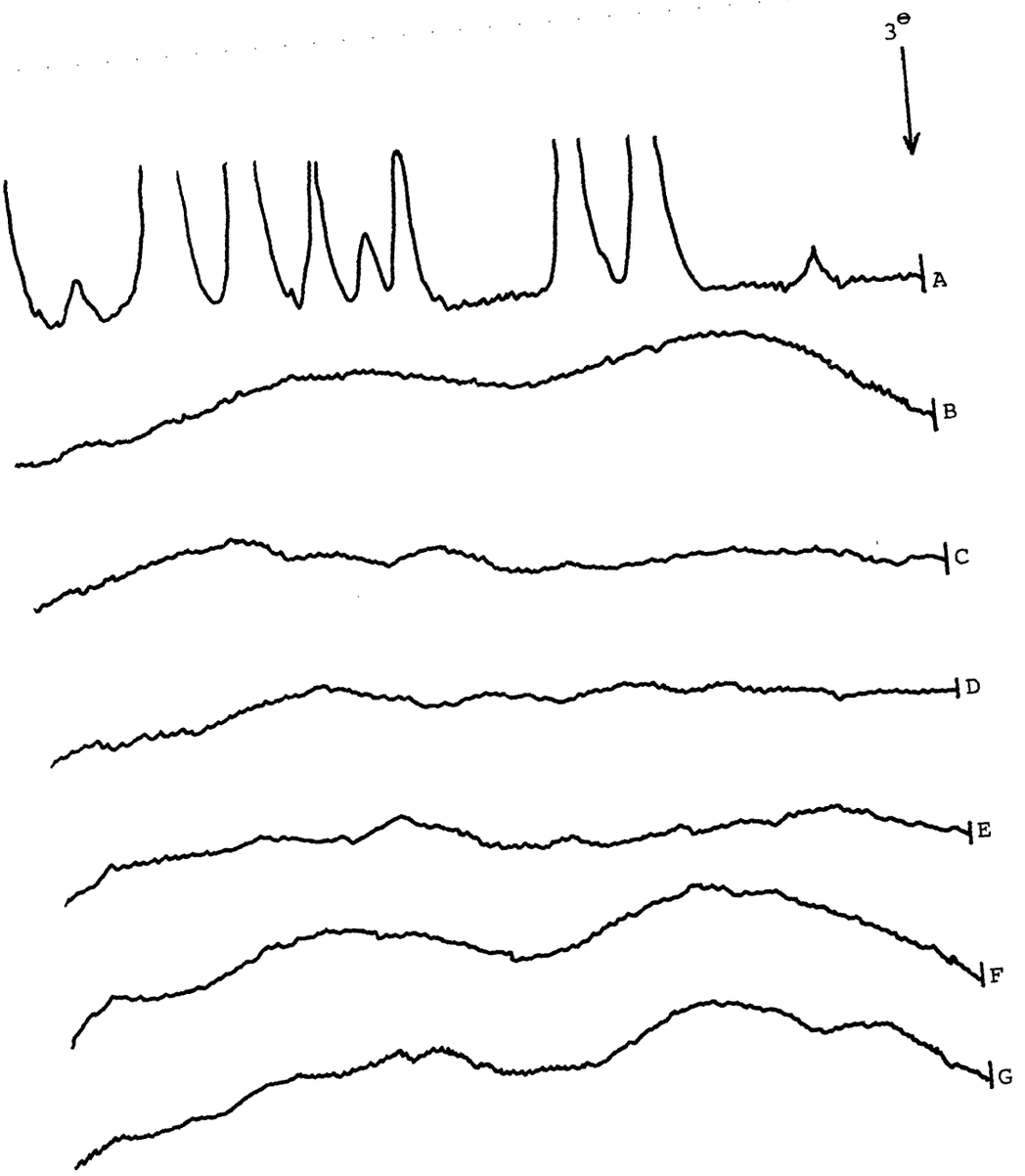


Fig. 2.13.2 X-Ray Diffraction Patterns For SSA (A), PVP (B), And SSA:PVP Coprecipitates 2:1 (C), 1:1 (D), 1:2 (E), 1:5 (F) And 1:10 (G).

indicated, as expected, that the crystalline structure of SSA could be detected over the amorphous nature of PVP as shown in Fig. 2.13.3. The progressive decrease in the SSA peak intensities could be related to increased proportion of the PVP present.

2.13.3 X-RAY DIFFRACTION EXAMINATION OF SSA:CA COPRECIPITATES AND ADMIXTURES

X-ray diffraction studies using a powder plate diffractometer indicated that, with the exception of the 1:1 molar ratio, SSA, CA and their coprecipitates were crystalline. The diffraction peaks of the coprecipitates were not coincident with those for SSA or cholic acid. Fig. 2.13.4 shows this for a representative coprecipitate. This indicates that the coprecipitates appear to have a different crystalline structure from either of the original components. Since the X-ray diffraction pattern of SSA has previously been shown not to be altered by its recrystallisation from methanol (See Fig. 2.3.5.1) it was concluded that changes in the X-ray diffraction patterns for the coprecipitates could be due to an alteration in the crystal structure of cholic acid following its recrystallisation from methanol. This possibility was examined by comparing the X-ray diffraction patterns for cholic acid, as supplied, to that after recrystallisation from methanol, and the resultant patterns shown in Fig. 2.13.5 indicate a change in the crystal structure.

The possibility of different polymorphic forms of cholic acid cannot therefore be discounted. Comparison of the diffraction patterns for the SSA:CA coprecipitates, that exhibited a crystalline behaviour, with cholic acid recrystallised

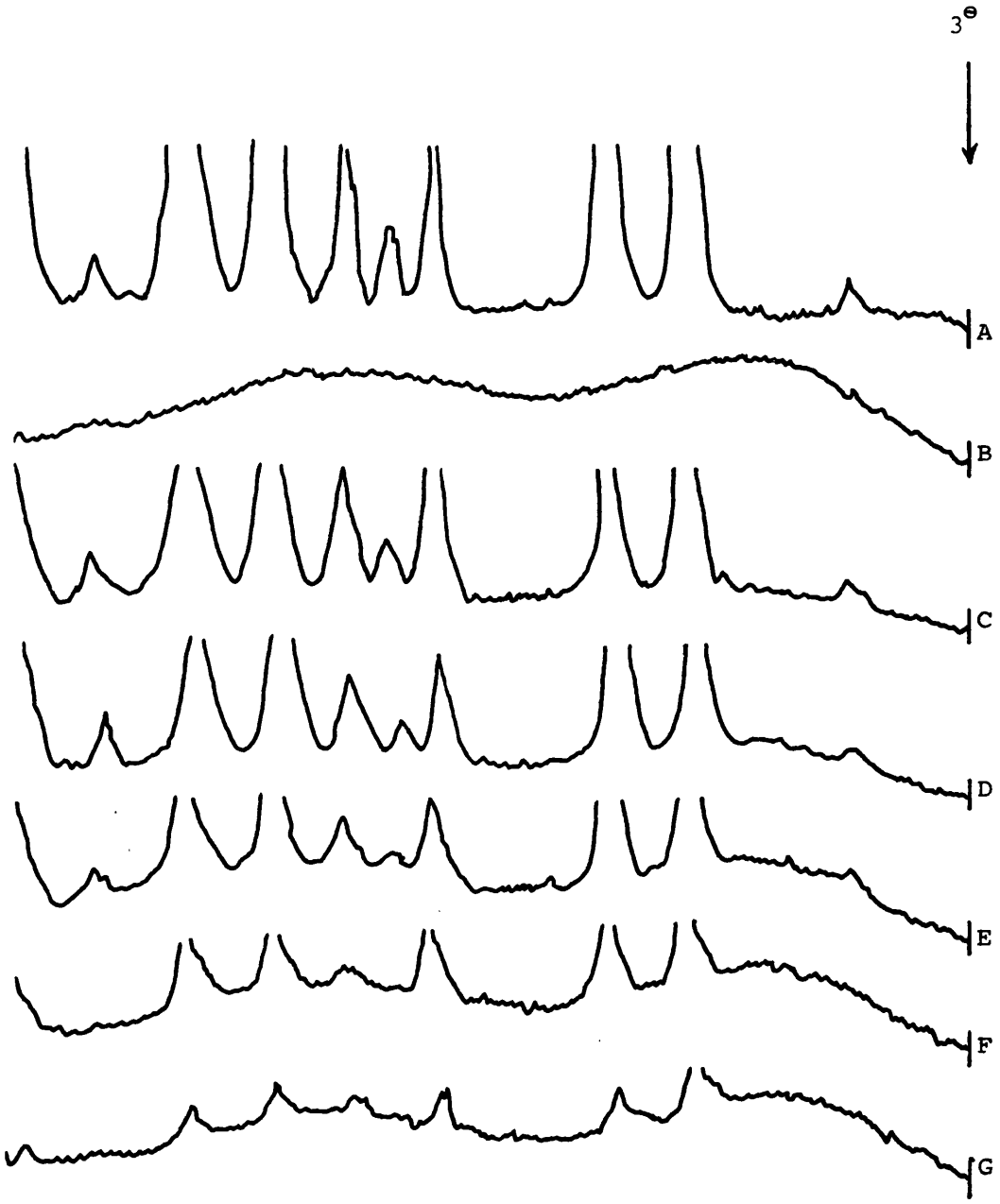


Fig. 2.13.3 X-Ray Diffraction Patterns For SSA (A), PVP (B), And SSA:PVP Admixtures 2:1 (C), 1:1 (D), 1:2 (E), 1:5 (F) And 1:10 (G).

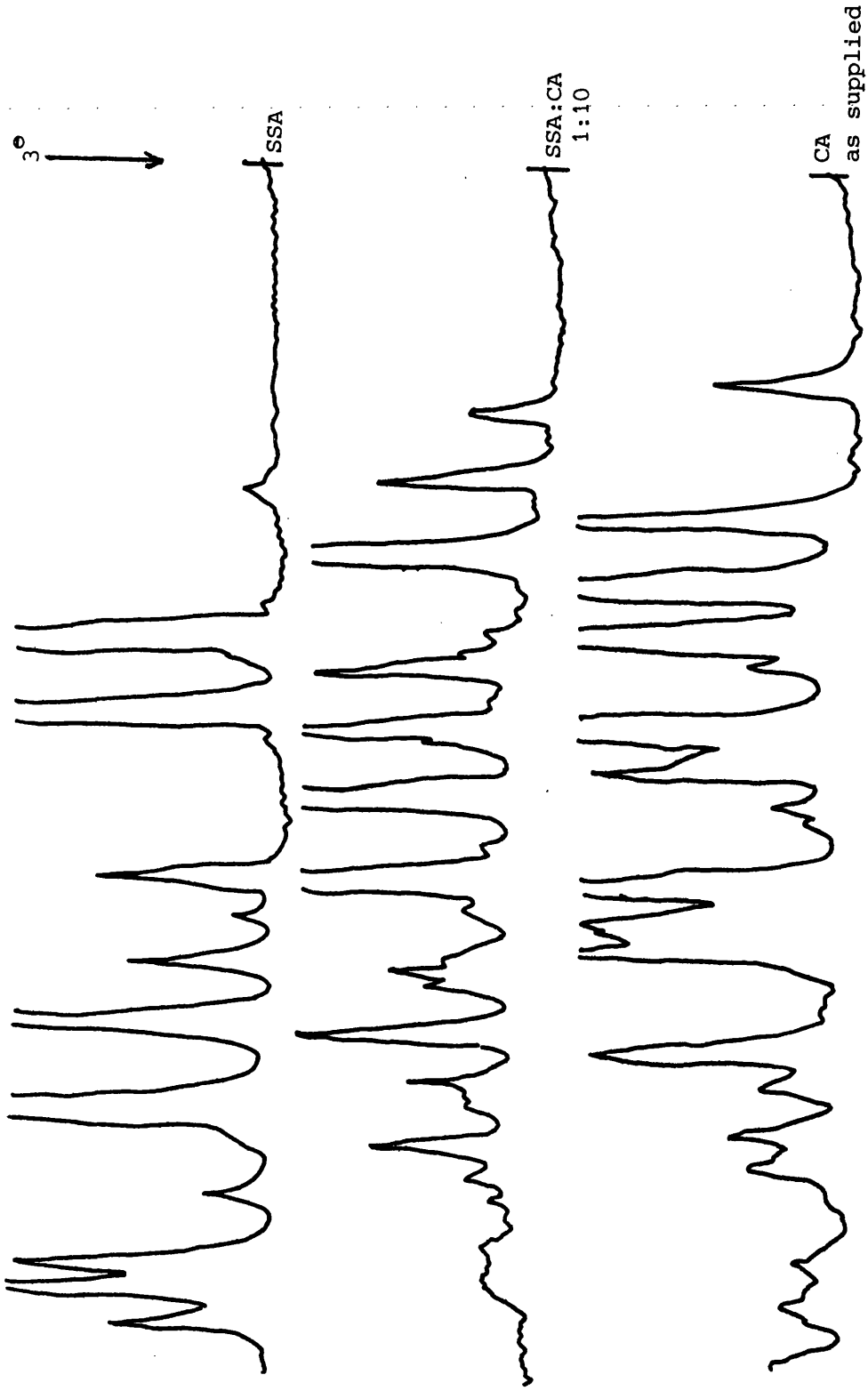


Fig. 2.13.4 Representative Diffraction Pattern Of The SSA:CA Coprecipitates In Comparison To SSA And Cholic Acid As Supplied.

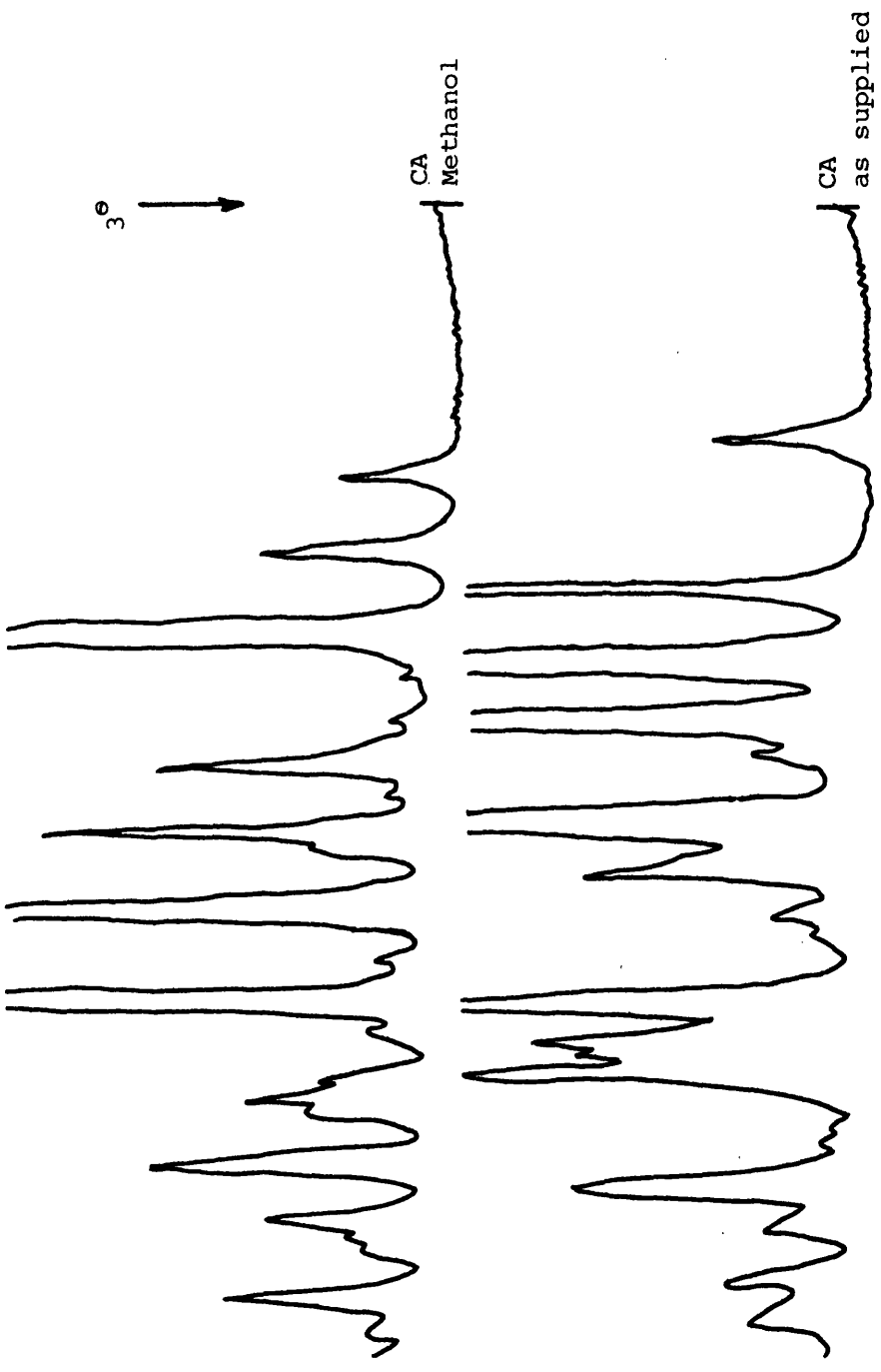


Fig. 2.13.5 X-Ray Diffraction Patterns For Cholic Acid As Supplied And After Its Recrystallisation From Methanol.

from methanol verifies that changes in their diffraction pattern are due to alteration of the crystal structure of cholic acid. Fig. 2.13.6 shows that all of the diffraction peaks for the SSA:CA coprecipitates can therefore be attributed to either SSA or cholic acid and not to any other product of crystallisation. The strikingly anomalous amorphous pattern for the 1:1 ratio and the poorly crystalline nature of the 1:2 ratio was checked by examination of the diffraction patterns of newly prepared samples. The original findings were confirmed and it was envisaged that the amorphous nature of the SSA:CA 1:1 and 1:2 coprecipitates might be reflected in their absorption behaviour.

In light of the observed crystal changes for cholic acid resulting from its recrystallisation from methanol it was essential that the correct crystalline form be used in preparation of the corresponding admixtures. Therefore, for reasons of comparison, the SSA:CA admixtures were re-prepared using cholic acid recrystallised from methanol and sieved before use.

The X-ray diffraction patterns for the SSA:CA admixtures show complete masking of the SSA peaks even for the 2:1 ratio. All peaks are characteristic of those for the methanol recrystallised cholic acid. A representative pattern is given in Fig. 2.13.7 for the SSA:CA 1:2 admixture with references of SSA and cholic acid.

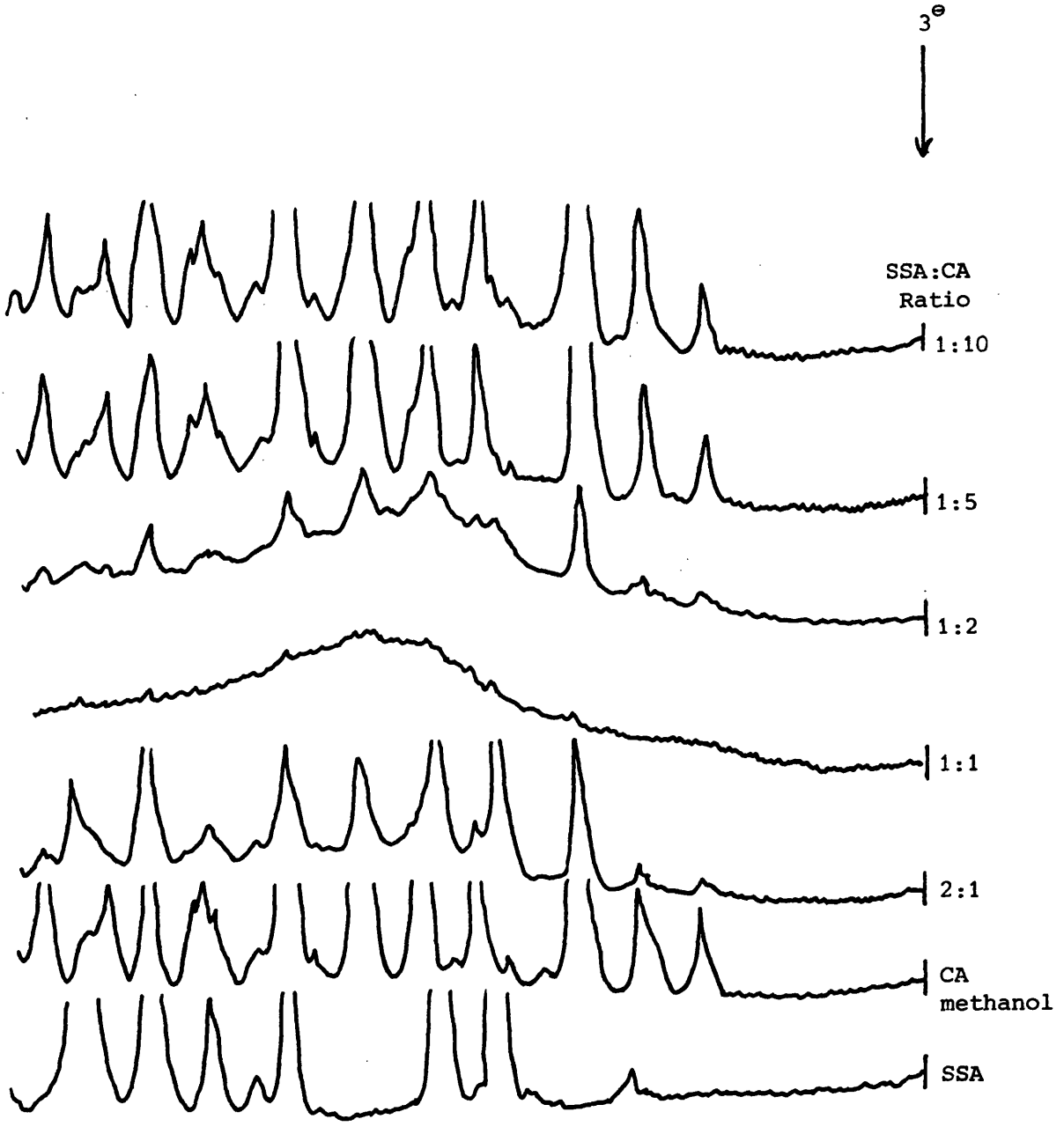


Fig. 2.13.6 X-Ray Diffraction Patterns For SSA:CA Coprecipitates, Cholic Acid Recrystallised From Methanol And SSA.

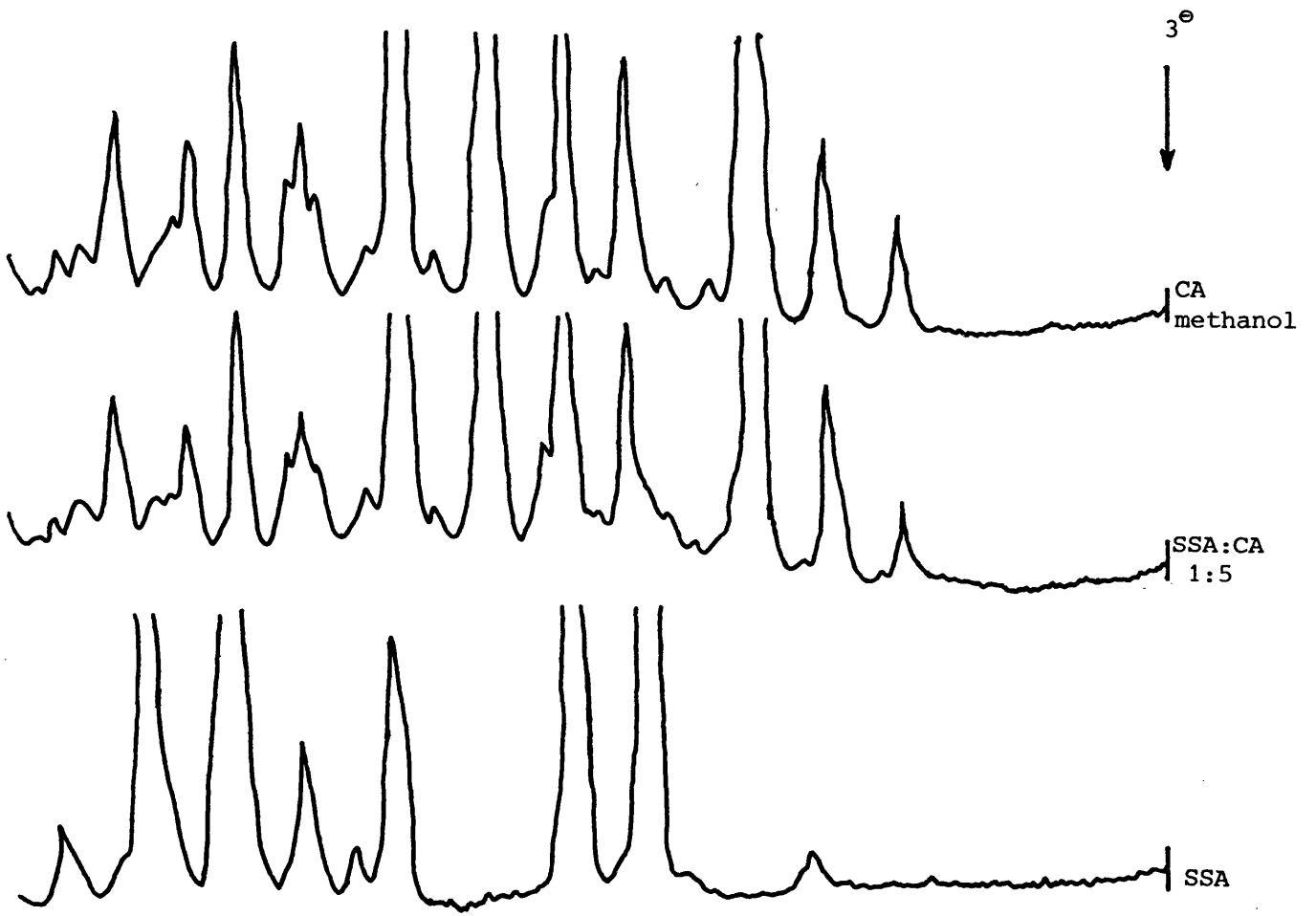


Fig. 2.13.7 Representative X-Ray Diffraction Pattern For SSA:CA Admixture (1:5 Ratio Shown), Cholic Acid Recrystallised From Methanol And SSA.

2.13.4 X-RAY DIFFRACTION EXAMINATION OF SSA:DOCA COPRECIPITATES AND ADMIXTURES

The powder plate X-ray diffraction technique was again used for the examination of the diffraction patterns of the SSA:DOCA coprecipitates and admixtures. Experience with the cholic acid systems suggested an examination for changes in the crystalline nature of deoxycholic acid following its recrystallisation from methanol prior to studies on the coprecipitates and preparation of the admixtures. Fig. 2.13.8 shows changes in the diffraction pattern of the methanol recrystallised deoxycholic acid. As for the SSA:CA admixtures, those for SSA:DOCA systems had to be prepared using deoxycholic acid recrystallised from methanol. Fig. 2.13.9 gives the diffraction patterns for the SSA:DOCA coprecipitates, and it is apparent, that changes in their crystalline nature can be attributed to alteration of the diffraction pattern of deoxycholic acid. Critical examination of Fig. 2.13.9 reveals that during progression through the series (SSA:DOCA molar ratio) from 2:1 up to 1:10 the associated changes in X-ray diffraction behaviour reflect the progressive change in the ratio of constituents. This is illustrated by the 2:1 and 1:10 SSA:DOCA ratios, where the former shows a diffraction pattern that is predominantly that of SSA whereas in the latter the emphasis is on the diffraction peaks attributable to deoxycholic acid. It should be noted that although the 1:2 ratio coprecipitate showed a poorly-crystalline diffraction pattern the 1:1 ratio had a pattern indicative of a definite crystal structure. This is in contrast to the respective SSA:DOCA coprecipitates. Furthermore, all SSA:DOCA coprecipitates exhibited an initial diffraction peak (labelled

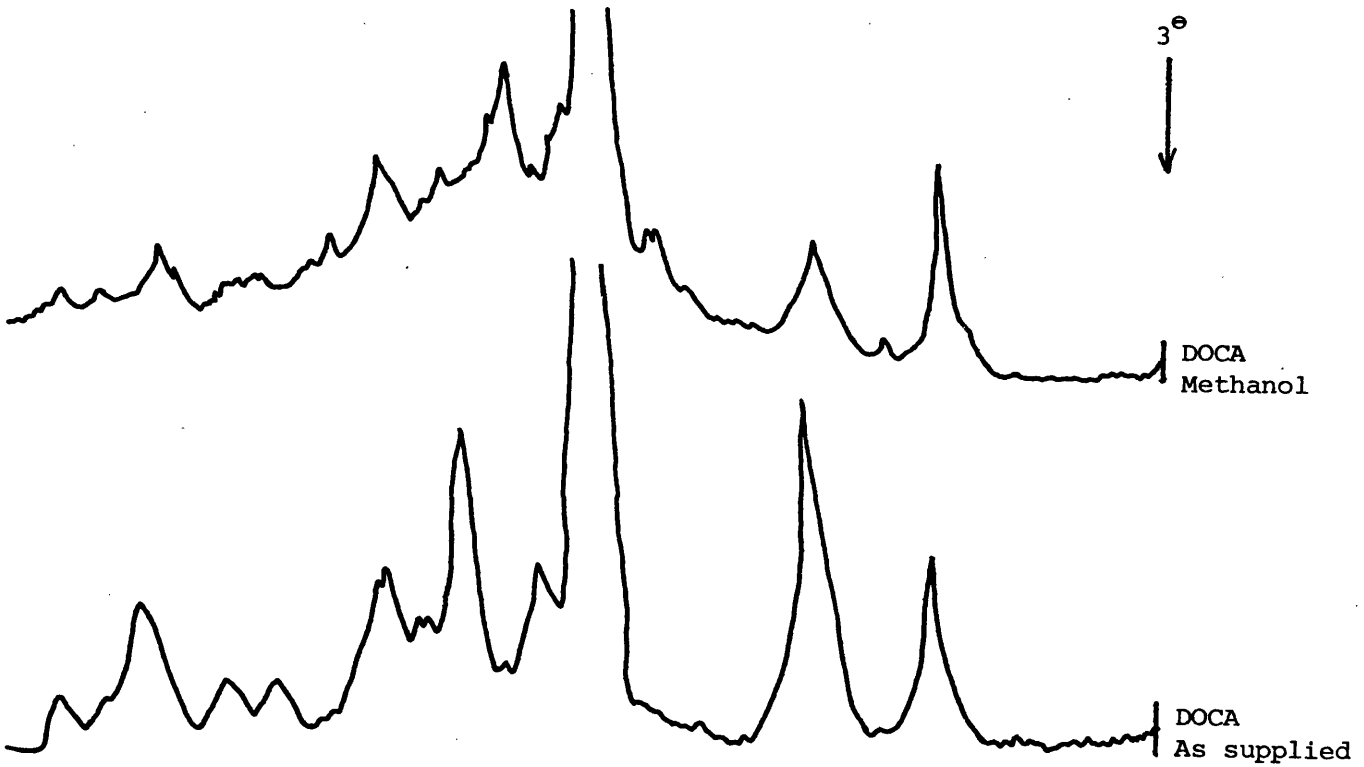


Fig. 2.13.8 X-Ray Diffraction Patterns For Deoxycholic Acid (DOCA) As Supplied And After Recrystallisation From Methanol.

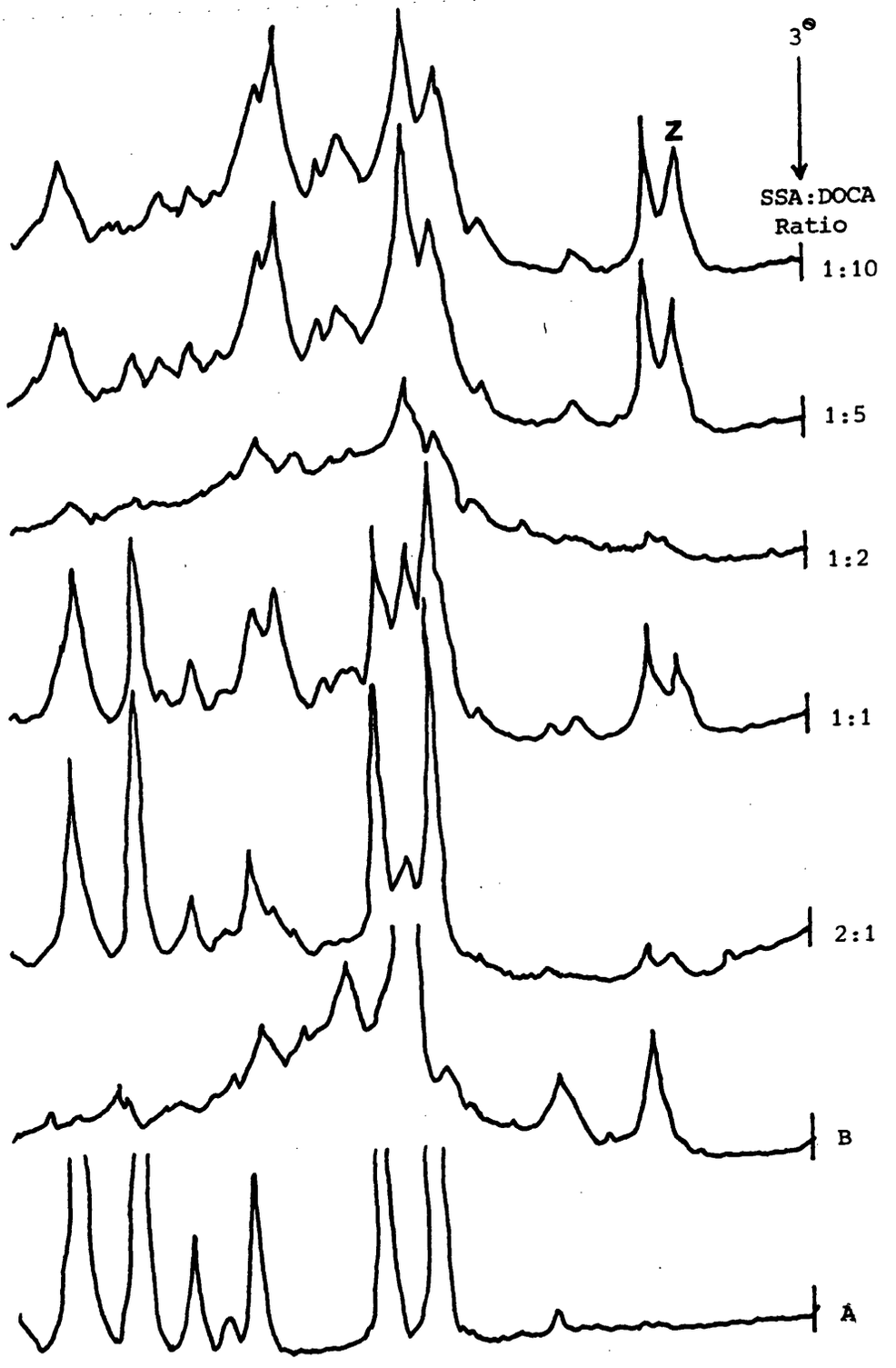


Fig. 2.13.9 X-Ray Diffraction Patterns For SSA:DOCA Coprecipitates, Deoxycholic Acid Recrystallised From Methanol (B) And SSA (A).

Z in Fig. 2.13.9) that could not be accounted for.

The X-ray diffraction patterns of the SSA:DOCA admixtures were composed of the peaks of the two crystalline and non-interactive components and reflected the ratio of SSA to deoxycholic acid. Representative diffraction patterns for the 2:1 and 1:10 SSA: DOCA admixtures are given in Fig. 2.13.10 with those of SSA and methanol recrystallised deoxycholic acid for reference.

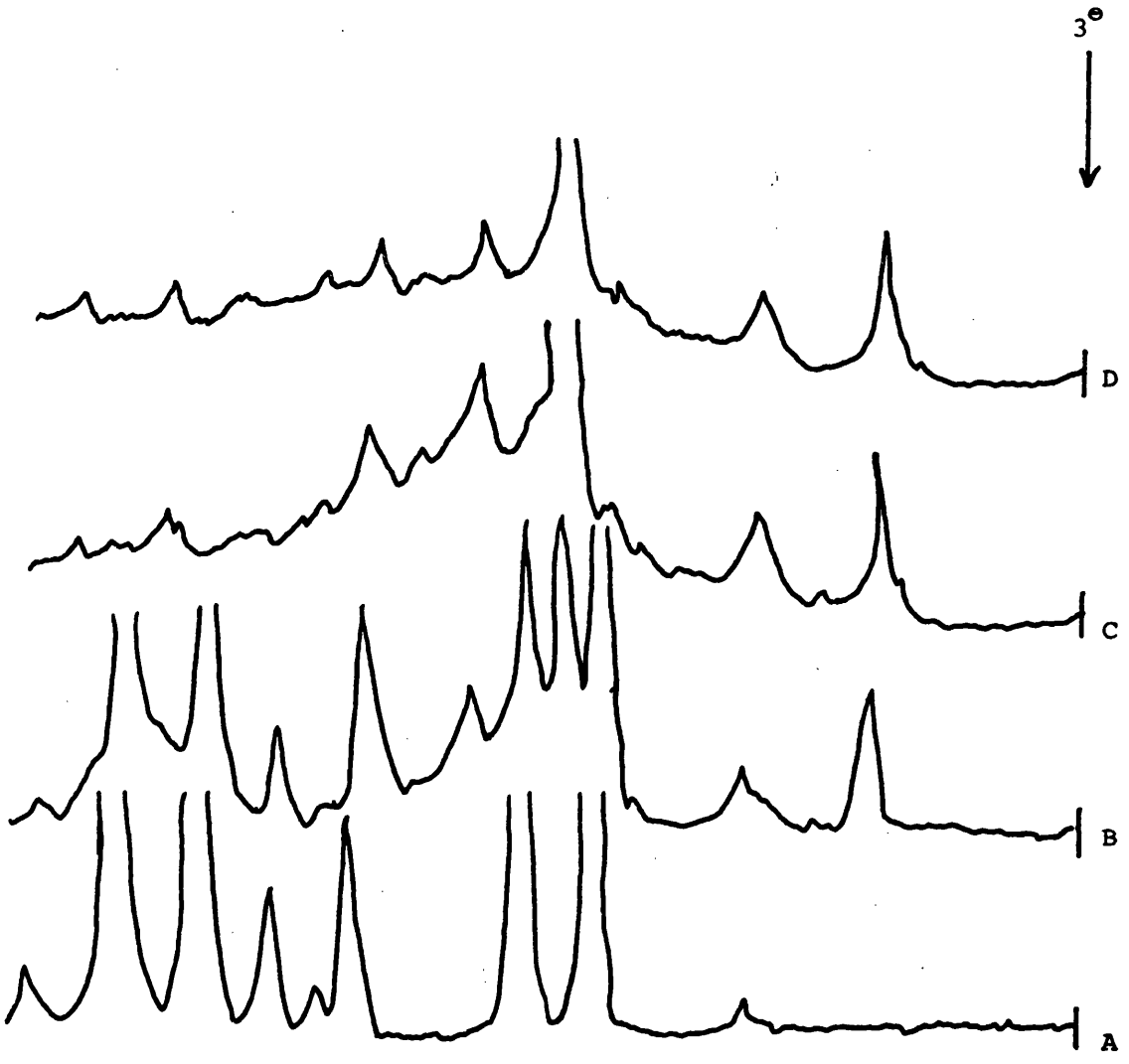


Fig. 2.13.10 X-Ray Diffraction Patterns For Two Representative SSA: DOCA Admixtures (B 2:1, C 1:10) Deoxycholic Acid Recrystallised From Methanol (C) And SSA As Supplied (A).

2.13.5 DISSOLUTION AND SOLUBILITY STUDIES ON SSA:CA COPRECIPITATES AND ADMIXTURES

It has been suggested that the amorphous nature of the SSA:CA 1:1 and the poorly crystalline nature of the 1:2 coprecipitates may be reflected in an enhancement of SSA absorption. This was found to be the case for the 1:1 coprecipitate and admixture but for no other SSA:CA system (See 3.3.17). The respective areas under the plasma salicylate concentration vs time profiles for the 1:1 systems were significantly greater than the other SSA:CA formulations and showed 100% bioavailability in comparison to the same dose given intravenously. The observed changes in bioavailability warranted explanation and, in an attempt to do this, the dissolution and solubility characteristics of the SSA:CA systems were assessed in vitro.

Dissolution Studies. The dissolution apparatus has been described in Section 2.11.1 and the experimental protocol remained unaltered except that the volume of the dissolution medium was reduced to 750 ml, since for some of the SSA:CA systems there was insufficient material to provide a saturated solution in the 1500 ml originally employed. As it was there was insufficient of the 1:5 coprecipitate remaining after the in vivo absorption studies to be examined in vitro and is therefore not included.

100 mg (equivalent SSA) of each SSA:CA system (75-105 μm particle size range) was added to 750 ml 0.1N HCl containing 5×10^{-3}% w/v polysorbate 80 stirred at 52 rev. $min.^{-1}$ and

maintained at 37°. Each system was corrected for % SSA content, according to Table 2.13.5 for the coprecipitates, whereas the ratio of the components in the admixtures were assumed to be as prepared. 5 ml samples were removed by filtration through a 0.45 μm millipore as previously described (See 2.11.1) and assayed for SSA accordingly.

The resultant dissolution profiles given in Fig. 2.13.11 show that dissolution for both the 1:1 coprecipitate and admixture is greater than that for the other SSA:CA systems and SSA alone (75-105 μm). It is also evident that all CA-containing systems show a greater amount of SSA in solution after 60 minutes than for SSA alone. For all systems studied, except the 2:1 coprecipitate, the material which settled during the experimental period formed a conical mound directly under the stirrer blade; the 2:1 coprecipitate was observed to form large aggregates that remained suspended. This 'caking' could therefore account for the anomalous profile that was produced by this material up to 45 minutes.

Solubility Studies. Saturation solubilities of the SSA:CA systems were determined using the experimental protocol previously described (See 2.6). Excess material was added to 0.1N HCl containing 5×10^{-3}% w/v polysorbate 80 and the equilibrium solubility determined after 24 hours. The results given in Table 2.13.6 show the presence of cholic acid to enhance the solubility of SSA by approximately 5% and that all CA-containing systems exhibited equivalent solubilities.

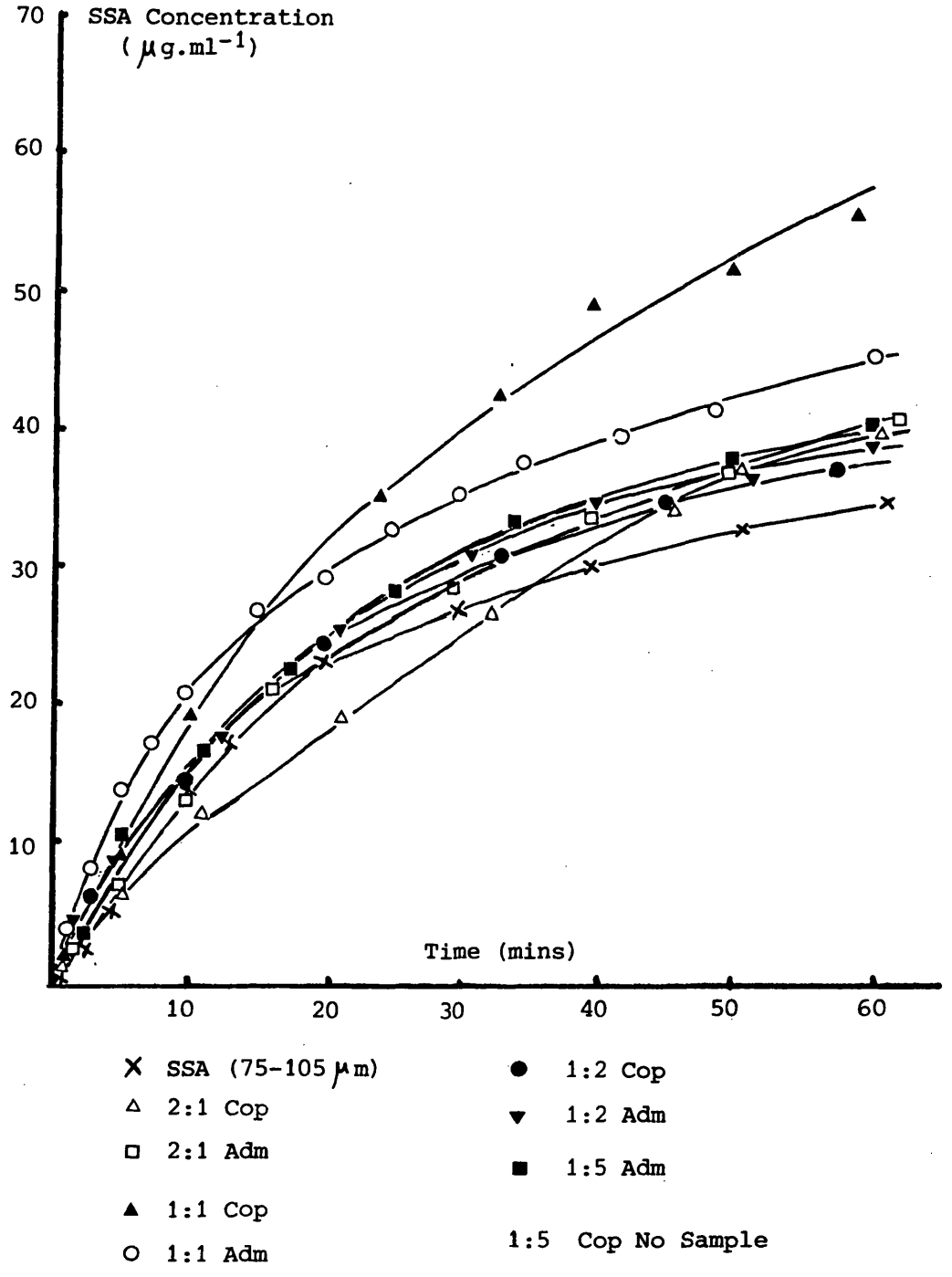


Fig. 2.13.11 Dissolution Profiles For SSA:CA Coprecipitates And Admixtures In 0.1N HCl Containing 5×10^{-3} w/v Polysorbate 80.

On the basis of the solubility studies the enhanced dissolution of the 1:1 SSA:CA formulations cannot be attributed to changes in solubility and may therefore reflect the amorphous nature of the 1:1 ratio coprecipitate (Fig. 2.13.6). For the 1:1 ratio admixture another possible mechanism must be proposed and may include a molecular interaction within the diffusion layer of the dissolving particles and/or the solubilization of SSA within cholic acid micelles/aggregates.

System	Absorbance (at 341 nm)	SSA concentration ($\mu g.ml.^{-1}$)	Mean
SSA alone	0.338 0.339	81.12 81.36	81.24
SSA:CA 2:1 COP	0.358 0.358	85.92 85.92	85.92
2:1 ADM	0.352 0.352	84.48 84.48	84.48
1:1 COP	0.348 0.353	83.52 84.72	84.12
1:1 ADM	0.349 0.350	83.76 84.00	83.88
1:2 COP	0.353 0.352	84.72 84.48	84.50
1:2 ADM	0.358 0.361	85.92 86.64	86.28
1:5 COP	0.368 0.365	88.32 87.60	87.96
1:5 ADM	0.368 0.370	88.32 88.56	88.56

Table 2.13.6 Equilibrium Solubilities of SSA:CA Coprecipitates And Admixtures In 0.1N HCl Containing $5x10^{-3}$% w/v polysorbate 80 at 37^{o}.

CHAPTER THREE

IN VIVO STUDIES

3.1 INTRODUCTION. SOME BASIC PHARMACOKINETIC CONCEPTS

The technical limitations associated with non-invasive methodology in biopharmaceutical and pharmacokinetic studies have meant that, for most drugs, assessment of their absorption and disposition relies on monitoring the drug concentration in body fluids and tissues. However, there are some drugs e.g. reserpine, that exert a clinical effect, the time course of which is not directly related to, and cannot be correlated with, blood or body fluid concentrations. Assessment of the absorption into and elimination from the body is employed, for the majority of drugs, to indicate the onset and duration of a clinically-useful or toxic response and to evaluate changes in that response resulting from pharmaceutical or formulatory variations.

Most absorption and disposition studies of drugs in the body depend on the measurement of unchanged drug and/or its metabolites in a body fluid (e.g. blood, urine etc) and are usually expressed graphically in the form of a concentration vs time profile. In recent years it has become possible to analyse these profiles by applying the principles of first order kinetics, an approach that has facilitated an understanding of the complex kinetic processes underlying the time course of a drug's presence in the body. The mathematical basis of this kinetic interpretation has been adequately reviewed by Notari (1975), Gibaldi and Perrier (1975) and Wagner (1971; 1975). This type of analysis is termed 'pharmacokinetics' and has been defined as "the study of the kinetics of absorption, distribution, metabolism and elimination of drugs and their pharmacol-

ogical, therapeutic or toxic response in animals and man". (Notari, 1975).

Implicit in any pharmacokinetic evaluation is the characterisation of a suitable model, or models, based on 'compartments' in the body exhibiting similar pharmacokinetic profiles. In this context a compartment may be defined as any 'kinetically-distinguishable pool'; it may represent a specific anatomical entity (e.g. liver or lung), a specific physiological entity (e.g. blood or urine) or a non-specific collection of entities (e.g. circulatory fluids, adipose or well-perfused tissues). The advantage of such compartmental analysis is that knowledge of the pharmacokinetic profile for one readily-accessible compartment enables prediction of profiles for other non-accessible compartments.

The transfer of drug or its metabolites from one compartment to another is associated with specific rate constants which are generally first order and independent of dose. However, zero order transfer is being reported with increased frequency for capacity-limited processes that may be described by Michaelis-Menten kinetics. Compartmental analysis, therefore, provides quantification of these rate constants together with estimates of the volume of the compartment into which the drug is distributed.

Pharmacokinetic analyses have been utilised experimentally to determine the influence of formulation on absorption, disease or disposition and in comparisons of drug homologues in

structure-activity relationships. The clinical use of pharmacokinetics as a diagnostic tool has been proposed, particularly in cardiovascular, hepatic and renal disease, since there is a direct connection between these diseases and the disposition of both exogenous and endogenous substances.

The initial stage in a pharmacokinetic analysis therefore, is to define the compartmental model that adequately describes the data. Normally the simplest model is chosen but, in view of a comparison between the calculated and experimental data, this model may be modified, either to make it more complex or to simplify it further. In all compartmental analyses it must be remembered that the body is an 'open' system since it is continually disposing of administered drugs by excretion and/or metabolism.

Following an intravenous bolus injection the blood concentration vs time profiles for the majority of drugs are biphasic in nature (see Fig. 3.1) and may be described by a biexponential equation:-

$$P = A.e^{-\alpha t} + B.e^{-\beta t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (38)$$

where P is the plasma concentration of the drug at time t. This model is known as the 'two compartment open model' (Scheme 1 Fig. 3.2) and α and β are complex functions of the micro-constants k_{12}, k_{21} and k_2, and generally represent the distribution and elimination phases respectively. A and B are the extrapolated intercepts derived from a plot of log P vs t and represent the hypothetical concentrations of drug in the central compartment necessary to produce the distribution and elimination profiles

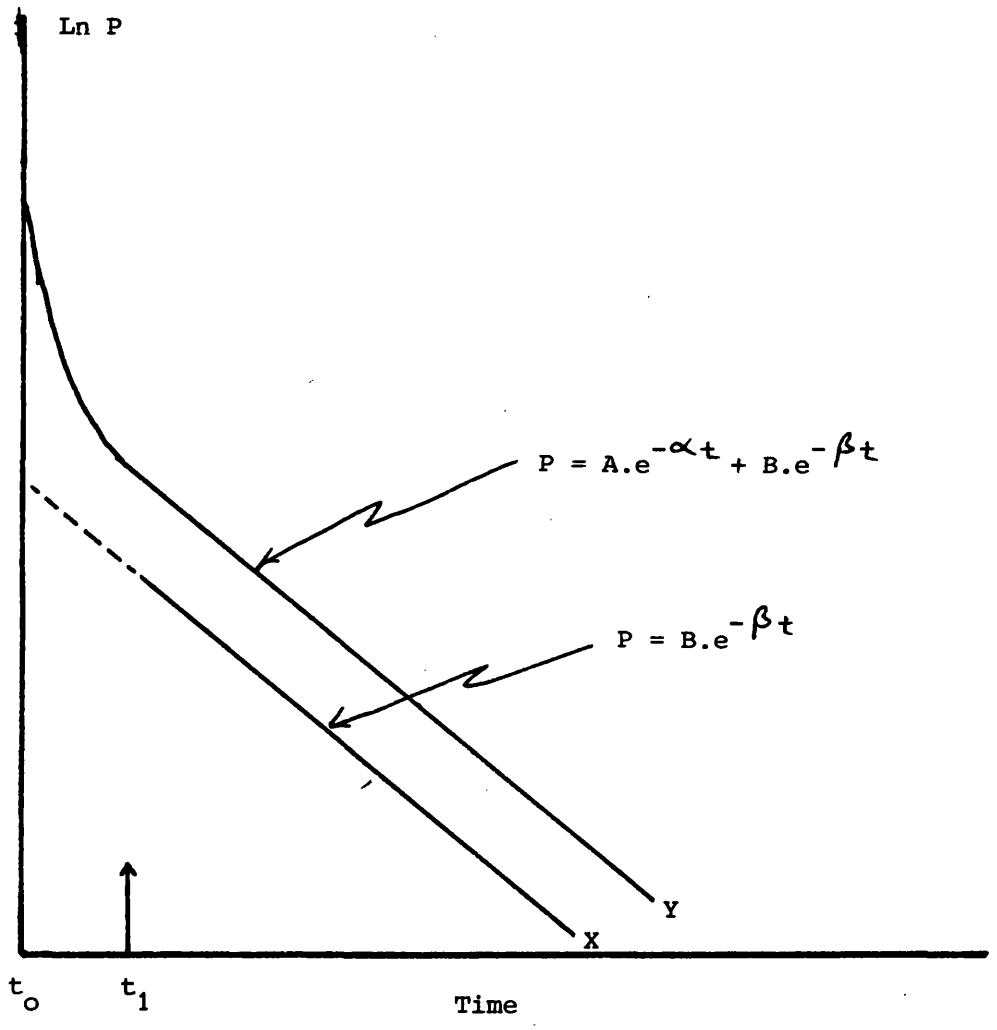


Fig. 3.1 Characteristic Profiles For A Plot Of $\log_e$ Of Drug Concentration In The Blood vs Time For Data Describable By A One (Line X) And A Two (Line Y) Compartment Open Model.

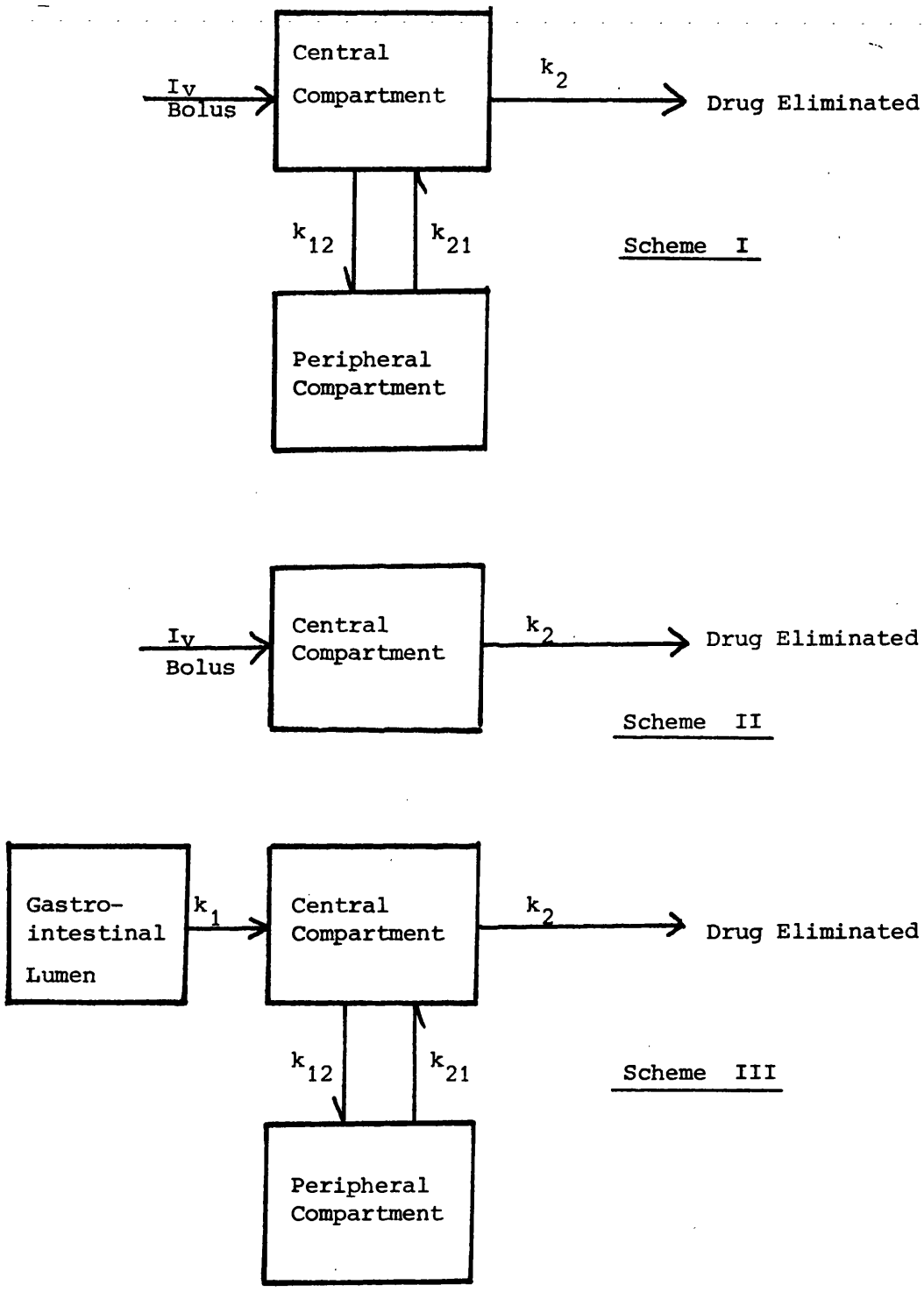


Fig. 3.2 Diagrammatic Representation Of Drug Distribution For The Two (Scheme I) And One (Scheme II) Compartment Open Model Following Intravenous Injection, And The Two Compartment Open Model For Oral Administration (Scheme III).

For explanation of the k values see text.

respectively.

Equation (38) has been presented and discussed by several authors (Mayersohn and Gibaldi, 1971; Riegelman et al, 1968) and the parameters, A, B, α and β can be determined by the method of residuals (feathering, stripping, peeling, back projection etc) as described by Notari (1975) and Wagner (1975). From a knowledge of these parameters the microconstants, k, can be calculated by use of equations given by the above authors. Although the method of residuals allows calculation of the microconstants, k, from α and β the elimination phase (β) must be adequately defined by the experimental data; in practice this entails collection of blood and/or urine samples for a time equal to seven-ten times the elimination half-life.

For some drugs, distribution occurs very rapidly with respect to elimination such that an equilibrium between the concentrations of drug in the central and peripheral compartments occurs in a very short time. In this case $\alpha \gg \beta$ and, if the blood concentration is not determined at sufficiently short periods following i.v. bolus injection, the pharmacokinetic profile may appear monophasic (See Fig. 3.1 line X) and may be described by the monoexponential equation (39).

$$P = B.e^{-\beta t} \qquad \text{..... (39)}$$

which has the same designations as in equation (38). This model, known as the 'one compartment open model', is shown in Scheme II Fig. 3.2. The simplicity of this model may, however, lead to misappropriation of the microconstants since the slope of the

monoexponential plot is not equal to k_2 as it would appear from the scheme, but always represents the overall elimination rate from the body, designated by β . Equation (39) does not imply that the distribution processes differ from those in the two compartment model, only that they occur very rapidly and therefore may not be detected experimentally.

The description of a drug's distribution according to the one- or two-compartment open model is therefore dependent upon the frequency of obtaining the initial blood samples and, under certain circumstances, it may be reduced from the two- to one-compartment model. This is illustrated by examination of Fig. 3.1 which shows that, if the first blood sample is taken at time t_1 following an intravenous bolus injection, then the data is described by a monoexponential equation (39), line X; whereas, if sufficient blood samples are taken between times t_o and t_1, the initial distributive phase may be detected and the data may then be described by a bi-exponential equation (38) line Y.

Drug administration via the intravenous route ensures that all of the administered dose is available to the circulatory fluids and the area under the i.v. plasma level profile (AUC_{iv}) is directly proportional to the dose. However, following oral administration the shape of the plasma concentration vs time profile described by Scheme III Fig. 3.2 will be influenced by the rate and extent of the drugs' availability, in addition to the influences of distribution and elimination discussed earlier. For Scheme III(Fig. 3.2) k_1 is defined as an availability (rather than an absorption) rate constant since it combines not only

the absorption process but also the rate of dissolution from a solid dosage form. Where only the rate of availability changes for a given drug in a single patient then the plasma concentration vs time profiles might appear as illustrated in Fig. 3.3.A. As k_1 increases the peak plasma concentration (P_p) will increase and the time taken to reach that peak (t_p) will decrease although, if the drug is completely absorbed before it passes the sites of its absorption from the gastrointestinal tract, the area under the plasma level profile (AUC_{po}) will be constant. Therefore for a given dose contained in various formulations, a constant value for the AUC is indicative that the same amount of drug was released from each formulation and subsequently absorbed. Furthermore, when k_1 is the only parameter undergoing change the AUC will be proportional to the total amount of drug released from the dosage form. Similarly if the size of the available dose is changed without any alteration to any of the pharmacokinetic microconstants then the AUC will be directly proportional to the dose. This would be true for oral as well as i.v. administration. This situation is illustrated for the case of oral administration in Fig. 3.3.B where peak concentration (P_p) of the drug occurs at the same time even though the concentration changes in a dose-related manner.

For drug disposition describable by the one compartment model the plasma concentration (P) is given by:-

$$P = \frac{fD}{V} \cdot \left(\frac{k_a}{k_a - \beta}\right) \left(e^{-\beta t} - e^{-k_a t}\right) \quad \ldots\ldots\ldots\ldots\ldots (40)$$

where fD is the fraction of the dose absorbed (assuming incomplete bioavailability from the site of absorption), V is

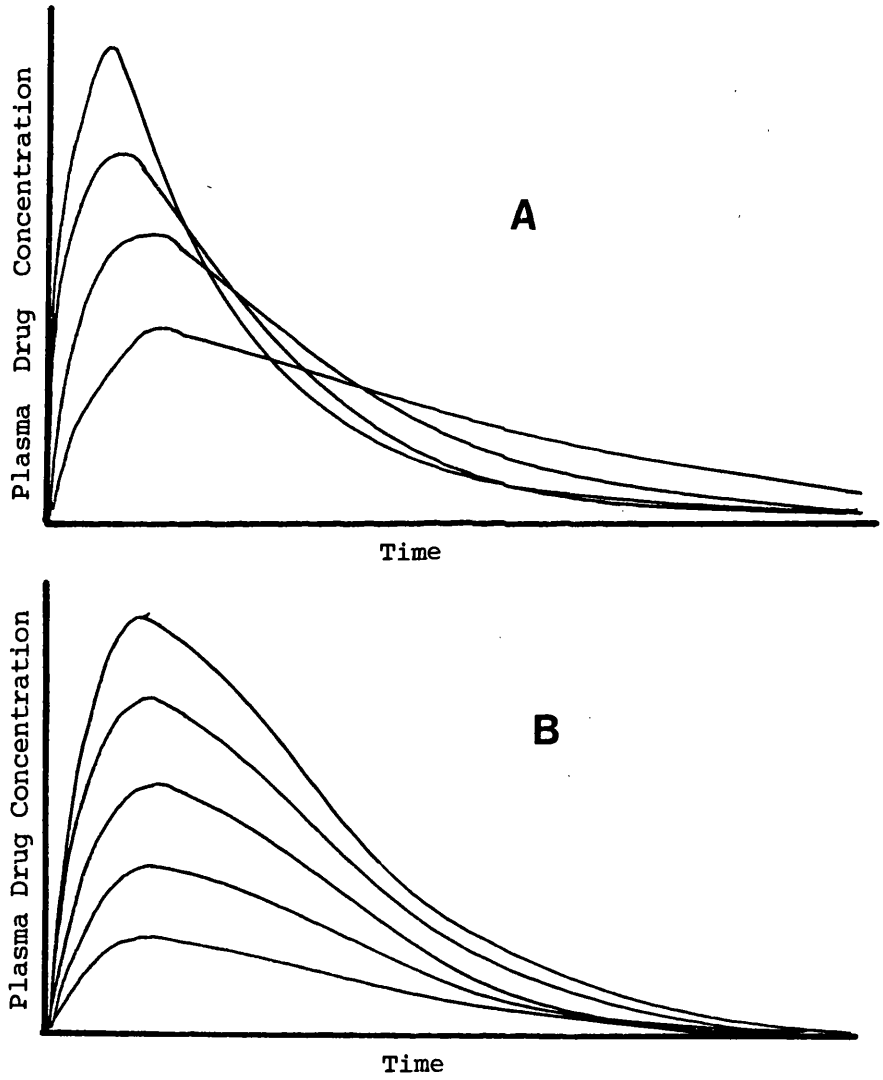


Fig. 3.3 Influence Of Alteration In The Absorption Rate Constant (A) And The Size Of The Dose Available For Absorption (B) From The Gastrointestinal Tract.

the volume of distribution and k_a is the apparent first order absorption rate constant. A plot of Ln P vs t will have a linear elimination phase (β) and application of 'stripping' procedures (Notari, 1975) will enable elucidation of k_a. Determination of k_a for the two compartment model (Scheme III Fig. 3.2) involves the incorporation of a term for k_a into equation (38) to give:-

$$P = Ae^{-\alpha t} + Be^{-\beta t} - (A + B)e^{-k_a t} \quad \ldots\ldots\ldots\ldots (41)$$

This is a complex situation where two exponential terms describe drug disappearance from the blood and one describes drug absorption. A usual assumption with this model is that $k_a > \alpha > \beta$ or $\alpha > k_a > \beta$, hence α is always greater than β. Calculation of k_a is based on the further assumption that all of the pharmacokinetic transfer and metabolic processes, excepting absorption, are first order.

The simplest approach to determination of k_a would be the measurement of drug loss from the site of absorption. Unfortunately in pharmacokinetic studies following oral or other extravascular routes of administration the amount of drug remaining unabsorbed is not usually available for direct measurement and values for k_a are derived from data describing the appearance of unchanged drug in the blood or urine. The most commonly employed methods for such inferential determinations are those of Wagner and Nelson (1963) and Loo and Riegelman (1968), for the one- and two-compartment models respectively. The applications of these methods are further discussed by Notari (1975) and Wagner (1974; 1975a; 1975b). For a single compartment where elimination is wholly describable by

capacity-limited, zero order, kinetics both of the above methods can be applied although this is not so where concomitant capacity-limited and first order elimination occur from either the one- or two-compartment open models.

Comparisons of blood level curves, for the same drug, may be summarised by two observations. A change in the time required to attain a peak plasma concentration, which reflects changes in k_a, and, a change in the area under the plasma level curve which indicates an alteration in the amount absorbed.

The early use of analogue computing techniques in pharmacokinetic analyses (Garrett, 1964; Garrett and Alway, 1964) has largely been superceded by the widespread availability of powerful digital computer procedures which together with the subjective nature of fitting data by analogue methods, has led to the development of 'pharmacokinetic modelling packages' that utilise non-linear optimisation methods to determine the micro-constants. The early SAAM package (Berman et al, 1962) was very cumbersome and has now been almost exclusively superceded by NONLIN (Metzler, 1969), a non-linear, least-squares regression program which has been rigorously tested and provides reliable least-squares estimates of the model parameters. The NONLIN programs have been incorporated into a package called AUTOAN (Sedman and Wagner, 1974) which has the provision for analysing zero-order transfer processes according to Michaelis-Menten kinetics. These programs require estimates of the parameters to be optimised, information that is normally provided by the method of residuals. However, the estimates of the parameters

should be well chosen in order that the optimisation procedures converge to a global and not a local minimum.

The overall elimination rate constant (β) of equations (38) and (39) is a composite function of terms that represent the loss of drug from the central compartment by excretion and metabolism. Excretion predominantly occurs via the kidneys into the urine but may also occur via the lungs, sweat, faeces (enterohepatic recycling) or saliva. Metabolic processes include chemical (hydrolysis, oxidation etc) or enzyme-catalysed degradation and transformation that may or may not be capacity-limited. If no capacity-limited (saturable) processes are involved in drug elimination then the apparent first order elimination rate constant (β or k_{e1}) is a summation of all the concomitantly-occurring first order elimination processes. However, if capacity-limited transport or metabolism occurs then the elimination phase will exhibit deviation from linearity and cannot be wholly described by equations (38) and (39) unless the contribution of the non-linear processes is negligible with respect to those of apparent first order. If the blood concentration is raised, by increasing the intravenous dose or the rate and extent of gastrointestinal absorption, then the contribution of the capacity-limited systems would be increased and k_{e1} (and the elimination half-life) will not be independent of the blood concentration.

When the processes of transfer associated with absorption, distribution, metabolism or excretion are described by first order kinetics then the biological half-life ($t_{\frac{1}{2}}$) will be

constant. In this context the biological half-life is the time required for the drug concentration in the central compartment to decrease to a half of the initial value, and as such should not be confused with the half-lives describing the absorptive or excretory processes. Since the determination of the biological half-life is dependent upon drug disposition obeying first order kinetics, any inconsistency in the half-lives calculated from data for different doses of a drug would be indicative that first order kinetics may not provide an adequate description of the blood drug concentration vs time profile. Therefore, changes in the biological half-life with respect to dose is one of the most discriminative methods of ascertaining the presence of capacity-limited transport or metabolic processes.

For drug transfer and metabolic processes that can be described by dose-dependent, zero order kinetics alone, the initial velocity of the process (v_o) can be described by the Michaelis-Menten equation:-

$$v_o = \frac{V_{max}.C}{K_m + C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (42)$$

where V_{max} is the maximum velocity of the process, C is the concentration of drug substrate available to the metabolic enzymes or membrane carrier system, etc and K_m is the Michaelis constant. A plot of v_o vs C will be a rectangular hyperbola characteristic of saturable concentration-dependent processes. At low concentrations the initial velocity will be first order

ie $$v_o = kC \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (43)$$

but at high drug substrate concentrations the velocity will be maximal, the reaction will be zero order, and V_{max} will be independent of drug substrate concentration. Furthermore, according to Michaelis-Menten kinetics, it can be shown that at half V_{max} the Michaelis constant (K_m) is equal to the drug substrate concentration.

Rearrangement of equation (42) gives

$$\frac{1}{\gamma_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (44)$$

This equation forms the basis of the Lineweaver-Burk method for estimating V_{max} and K_m since a plot of $1/\gamma_o$ (where γ_o is the overall zero order elimination rate constant obtained from a plot of blood drug concentration vs time) against $1/C$ (where C is the dose) will be linear, having a slope of K_m/V_{max} and ordinate and abcissa intercepts of $-1/K_m$ and $1/V_{max}$ respectively. Zero order elimination from a two-compartment open model is illustrated in Scheme IV(Fig. 3.4)

A combination of two simultaneously-occurring modes of elimination, one of which is capacity-limited, and the other first order, is given in Scheme V(Fig. 3.4) for the two-compartment open model. In this example elimination occurs from the central compartment by urinary excretion and metabolism, where the capacity of the enzyme system involved in the metabolic process may be saturable, resulting in deviation of the pharmacokinetic profile from the biphasic curve associated with the two compartment open model (See Fig. 3.5.Y). Under such conditions a plot

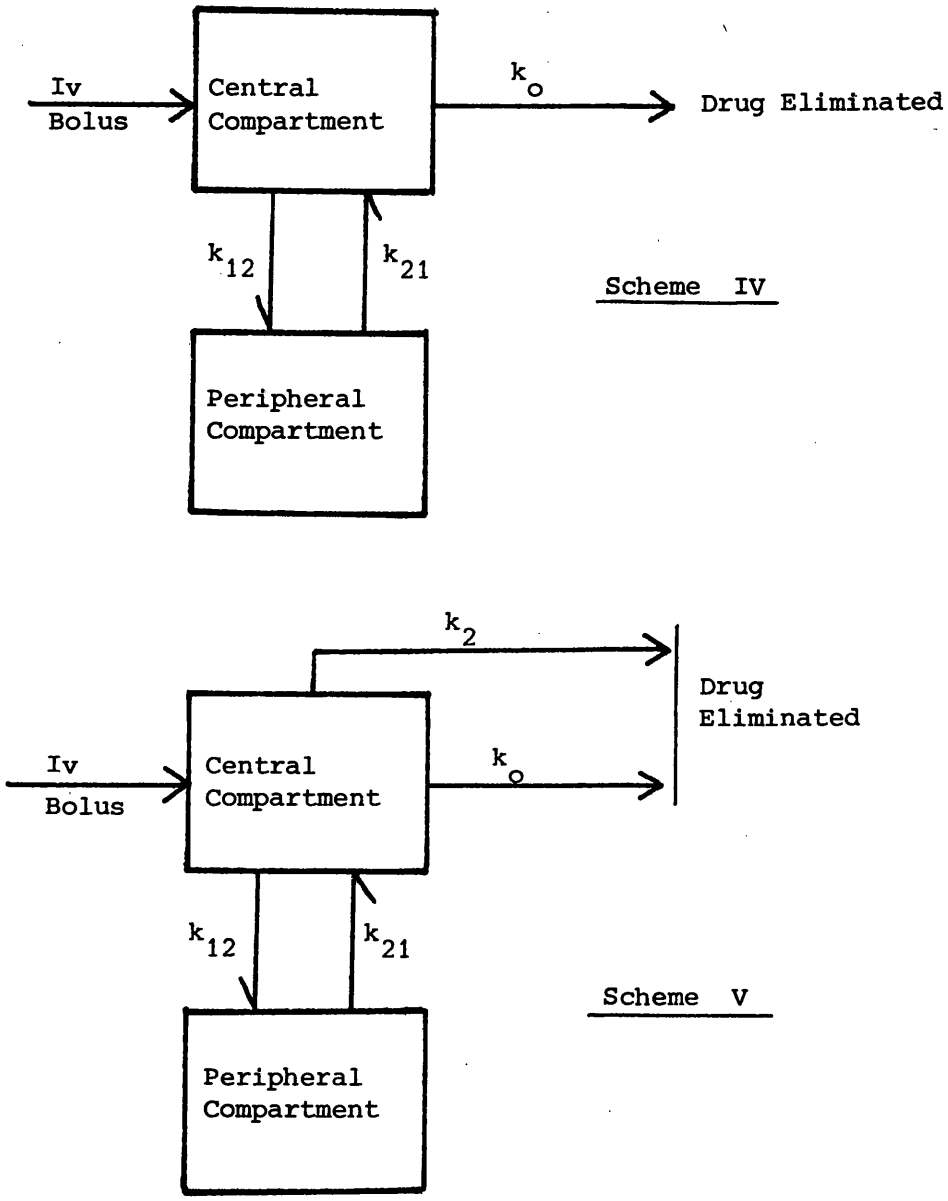


Fig. 3.4 Diagrammatic Representation Of Drug Distribution For The Two Compartment Open Model For Zero Order Elimination Kinetics (Scheme IV) And Parallel First And Zero Order Elimination Kinetics (Scheme V) (k_o and k_2 are zero and first order elimination processes respectively).

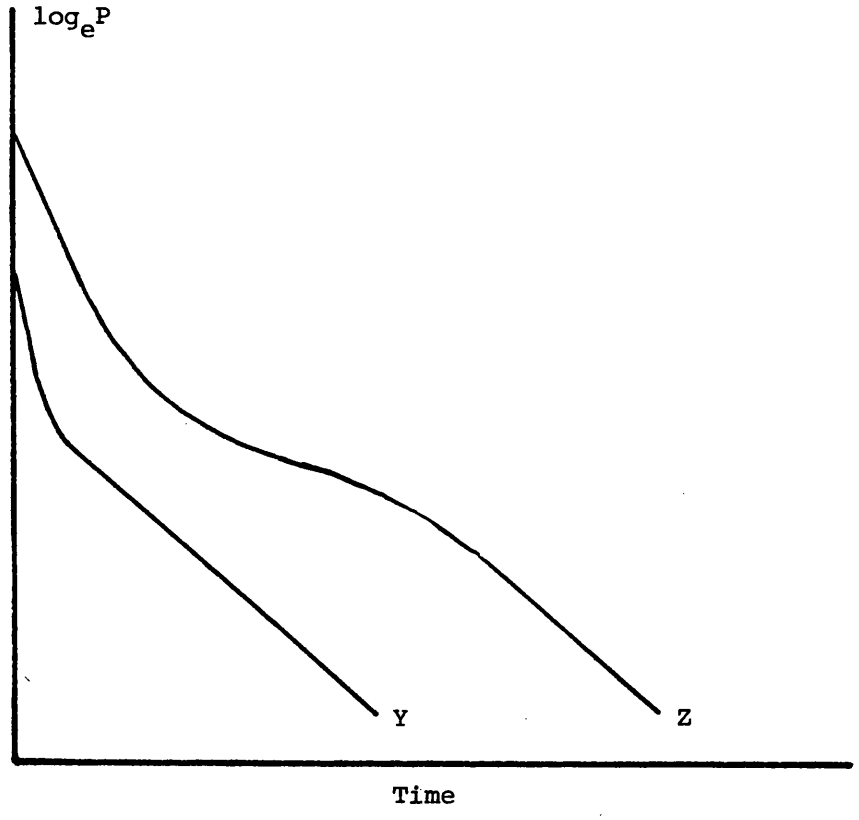


Fig. 3.5 Characteristic Profiles For A Plot Of $\log_e$ Of Drug Concentration In The Blood vs Time For A Two Compartment Open Model Describable By First Order Elimination Kinetics Alone (Line Y) And By Parallel First And Zero Order Elimination Kinetics (Line Z).

P vs time would appear as in Fig. 3.5 Line Z.

Capacity-limited, non-linear pharmacokinetic processes associated with drug absorption, distribution, metabolism, renal and biliary excretion, and pharmacological action have been collated and reviewed by Wagner (1973b). The majority of non-linear processes occurring during absorption and distribution are associated with physiological functions such as changes in blood supply and saturable tissue binding. However, by far the most convincing evidence for non-linearities is that dealing with the saturable metabolism of ethanol, phenytoin and the salicylates. Furthermore it has been suggested (Wagner, 1973b) that the parameters V_{max} and K_m will provide a better description of the saturable metabolism of these drugs, at all doses, than a series of zero order rate constants and elimination half-lives that vary with the amount of drug reaching the systemic circulation. Salicylate metabolism is of direct relevance to the work reported and will be discussed in greater detail.

Practically all of an administered dose of salicylic acid can be recovered from the urine as unchanged drug and as the four major metabolites, gentisic acid (GA), salicyluric acid (SUA), salicylacyl glucuronide (SAG) and salicylphenolic glucuronide (SPG). At low doses the major metabolite, salicyluric acid, has been shown to account for 0-91% of the salicylic acid dose administered (Schachter and Manis, 1958; Levy, 1965), the percentage decreasing as the dose was increased. The remainder of the dose is excreted in the approximate proportions:- GA, $<1\%$; SAG, 0-10%; SPG, 12-30%. The dose-dependent kinetics

of salicylic acid metabolism have been attributed to the processes which lead to the production of SUA, SAG and SPG (Levy, 1965; Levy et al, 1972) and have been suggested to account for the non-linearity of salicylate pharmacokinetics in man.

The precise site of formation of the various metabolites has not been identified although the indications are that the majority occurs in the liver. It has been suggested (von Lehmann et al, 1973) that the renal contribution of SUA formation could comprise 60-70% of the total metabolism of salicylic acid in man. This work was based on previous observations in the Rhesus monkey which clearly showed that the kidney was a major site for SUA formation (Wan and Riegelman, 1972). However, these conclusions are not supported by Lowenthal et al (1974) who demonstrated that the overall elimination rate of salicylate in anephric patients was no different to that in normal healthy subjects. The saturable nature of SUA formation in both man and the rat has been shown (Nelson et al, 1966) to result from enzyme saturation rather than the rate of glycine availability. The degree of saturation in both species was such that the percentage of the dose metabolised to SUA decreased with increasing dose and could therefore be expressed according to Michaelis-Menten kinetics. Although SUA formation is capacity-limited, its elimination in man follows first order kinetics, and this has been demonstrated following both oral and intravenous administration of SUA (Levy et al, 1969).

The possible salicylate moieties appearing as various metabolites

and unchanged drug in the urine, following oral administration of SSA are given in Fig. 3.6 with the metabolic pathways that have been shown to be capacity-limited. SSA has been detected in the urine following oral doses of 4-12 g (Hanzlik and Presho, 1925) and in a private communication B. K. Martin has reported the presence of SSA conjugates with glycine in the urine. In a crossover design pharmacokinetic study in elderly patients, that compared the absorption and elimination profiles for oral doses of SSA, aspirin and sodium salicylate, Nordqvist et al (1965) reported SSA elimination to have a longer half-life than that for aspirin and quoted approximate values of 7.8 and 4.7 hours for SSA and aspirin respectively.

The increased incidence of reports of drug disposition showing capacity-limited kinetics has produced a number of mathematical and graphical methods for obtaining estimates of the various pharmacokinetic parameters involved. All of the methods reported have relied on the assumption that in the absence of zero order kinetic processes, the drug concentration-time profile can be described by first order kinetics according to a one-compartment open model (Wagner, 1973a; Martis and Levy, 1973; van Ginneken et al, 1974). Wagner (1975) has indicated that analysis of the two-compartment open model with capacity-limited elimination kinetics is complicated by the difficulties of obtaining initial estimates of the parameters and the expense (in terms of computer time). In all reported methods for the one-compartment model initial estimates of the kinetic parameters are obtained by graphical interpolation and provides the basis for subsequent iterative procedures on a digital computer. The assumptions

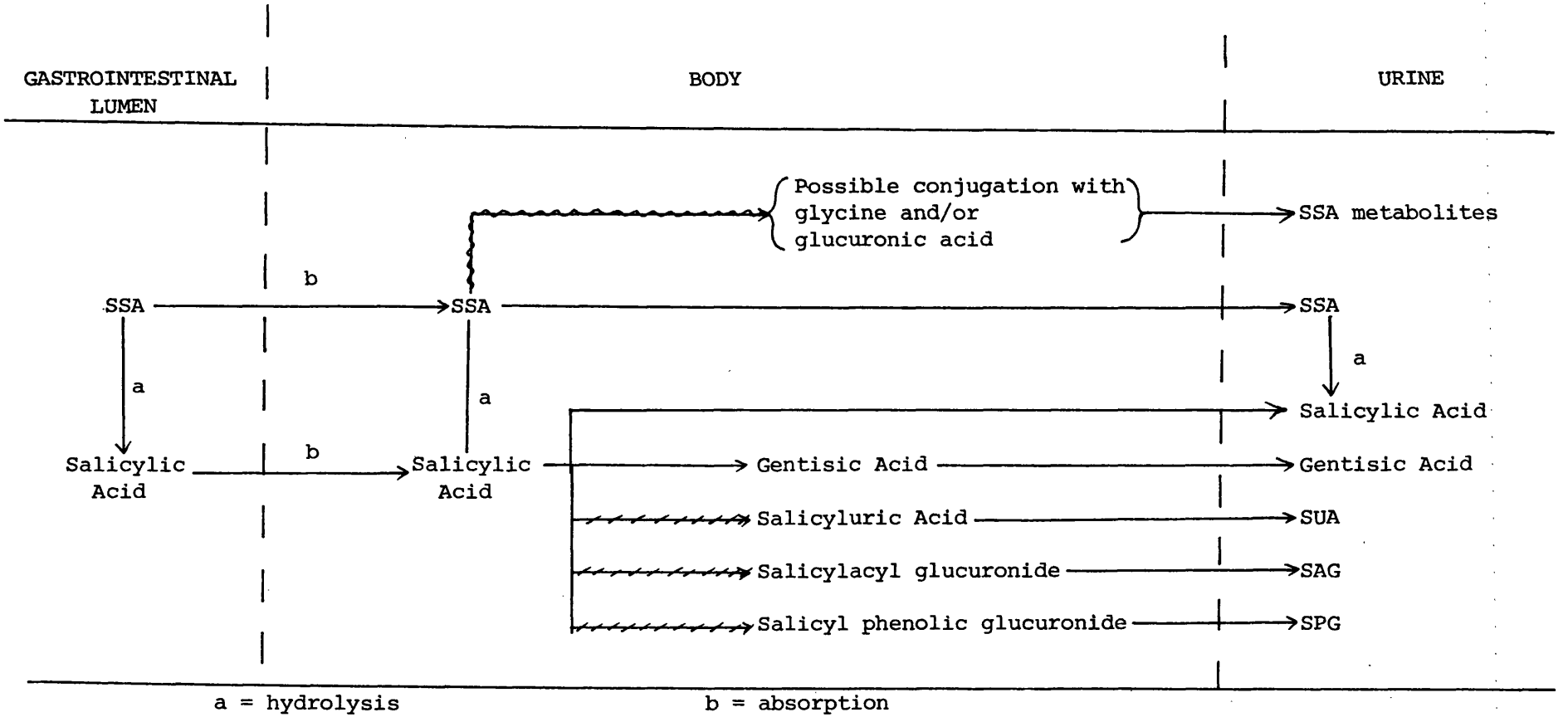


Fig. 3.6 Scheme For SSA Absorption, Metabolism and Excretion

made in any such analysis are that the values for K_m and V_{max} are invariant and that only one capacity-limited process is involved.

The pharmacokinetic description of drug absorption, disposition and elimination has, until now, been associated with the measurement of drug in the blood or plasma. Certain experimental or ethical considerations may preclude the regular or repetitive withdrawal of blood by venupuncture and, under such circumstances, other body fluids have been used to supply pharmacokinetic information. Next to blood the urine is the most commonly sampled body fluid and urinary excretion data may be used to monitor the elimination and absorption of a drug and/or its metabolites. The provision of information from urinary excretion of unchanged drug and/or metabolites can be used to discriminate between rates of absorption although, it has been shown to be less definitive than corresponding plasma level data (Levy and Yacobi, 1975). The interpretation of urinary excretion data has been the subject of a paper by Martin (1967) who compared the advantages and disadvantages of the two standard methods of analysis, the 'rate method', and the 'Sigma-minus method'.

The 'rate method' necessitates obtaining urine samples at frequent, equally-spaced time intervals and when the log. of the excretion rate for a given time interval is plotted against the mid-point of that time interval the slope should be negative and linear. Linearity of the slope is indicative of an unchanging excretion rate and is therefore particularly applicable to studies

where there is any progressive change in urinary excretion due to alterations in urine pH, volume, enterohepatic cycling or capacity-limited metabolism etc. The validity of this method does not depend on a knowledge of the total amount of drug excreted and, the loss of one urine sample does not invalidate the analysis.

The 'Sigma-minus method' consists of plotting the logarithm of the amount unexcreted $\left(\log (U_\infty - U_t) \right)$ *vs* time, where $(U_\infty - U_t)$ is the sum (sigma) of the amounts of drug excreted until such time as excretion may be considered complete (U_∞), minus the cumulative amount of drug excreted to a time t (U_t). When elimination is first order this is expressed as:-

$$\log (U_\infty - U_t) = \log U_\infty - \frac{k_t}{2.303} \quad \ldots\ldots\ldots\ldots\ldots\ldots (45)$$

and a plot of $\log (U_\infty - U_t)$ *vs* time will be linear with a negative slope of $-\frac{k}{2.303}$, where k is the overall elimination rate constant. Following oral administration there will be an initial time period over which such a plot will deviate from linearity due to absorption and can be used therefore to obtain information regarding the rate of drug absorption. The accuracy of the 'Sigma-minus' method depends entirely on the collection of urine until such time that excretion is complete and requires accurate determination of U_∞. Slight errors in estimating U_∞ will produce plots of $\log (U_\infty - U_t)$ *vs* t that appear to be linear for an initial period and then exhibit curvature in the terminal segments. If curvature (either +ve or -ve) occurs it is difficult to establish the origin of the

deviation and to ascertain whether it is a feature of the drug's elimination or an artifact resulting from incomplete urine collection.

The various models proposed for drug disposition shown in Figs. 3.2 and 3.4 describe the distribution of non-protein bound material between the constituent compartments and subsequent urinary excretion and metabolism. Inference of drug concentration in the body has been achieved by studying the appearance of unchanged drug and/or metabolites in the urine by the methods of analysis outlined above. However, the measurement of drug concentrations in the saliva is currently receiving increased attention as a tool for reflecting free drug concentrations in the systemic circulation and may therefore, be of use in bioavailability studies, monitoring of therapeutic drug concentrations and diagnosis of drug overdose, etc.

Significant correlations between plasma and saliva concentrations have been established for digoxin (Joubert et al, 1976), antipyrine (Brooks et al, 1976), salicylic acid (Graham and Rowland, 1972), paracetamol (Glynn and Bastain, 1973), theophylline (Koysooko et al, 1974), lithium (Groth et al, 1974), tolbutamide (Matin et al, 1974) and phenytoin (Bochner et al, 1974). The clinical applicability of using salivary drug levels has been suggested by most of the above authors and utilised for para-aminosalicylic acid (Krakowka et al, 1966) theophylline (Levy et al, 1974; Eney and Goldstein, 1975) and isoniazid (Boxenbaum et al, 1975).

The use of saliva drug concentrations in absorption studies could therefore provide a non-invasive method for following the time course of drug absorption and disposition that does not require medical supervision and allows the subjects to provide samples away from the laboratory. It is essential however, that a correlation between saliva and plasma concentrations of the drug should be established before the saliva can be routinely used as an inferential measure of blood concentration. This usually requires the measurement of free drug in the plasma since it is only that fraction of the total plasma concentration that is able to diffuse across the epithelia of the salivary glands. It has been shown that the diffusion of salicylate across the parotid epithelium of the dog was influenced by saliva pH but independent of saliva flow (Borzelleca and Putney, 1970) this study further related the change in saliva pH with plasma pH fluctuations associated with the intravenous infusion of hydrochloric acid and sodium bicarbonate. The contrasting experimental conditions employed by Borzelleca and Putney (1970) and Graham and Rowland (1972) have been tentatively suggested by the latter authors to account for the changing saliva/plasma salicylate ratio with time in the anaesthetised dog on the one hand, and the constant ratio observed in man on the other. Furthermore, since the saliva/plasma ratio is a function of the free drug concentration in the plasma it can be anticipated that an increase in the amount of free drug, resulting from higher blood levels and saturated binding, will be reflected by a corresponding decrease in the saliva/plasma ratio.

The evaluation of drug absorption using saliva can be further

complicated by contamination of the saliva with foodstuffs that are known to interfere with the drug assay. It should also be emphasised that several types of oral dosage forms, particularly solutions, suspensions, emulsions and uncoated tablets could lead to contamination of saliva resulting in erroneously high measured concentrations especially in the early samples of any study (Chiou et al, 1976; Joubert et al, 1976).

For estimation of drug bioavailability the extent of oral absorption is compared to that for an equivalent dose given by intravenous bolus injection, or if this is not feasible, to a standard reference oral dose which is usually a solution. The measured bioavailability for an orally-administered dose is an absolute term when compared to the corresponding intravenous dose, and a relative term when the reference is an oral solution, suspension, tablet or proprietary product. In the absence of urinary excretion data the extent of drug absorption by either route can be assessed from a knowledge of the area under the plasma level curve (AUC), which can be calculated by integration, to infinite time, of the equation that describes the drug concentration vs time profile. If the relevant equation is not known then the area, or a parameter associated with it, can be calculated by three methods. The most commonly employed technique is that of dividing the plasma concentration vs time profile into a series of trapezoids and calculating the sum of the areas for each trapezoid; this is easily achieved by computer techniques and a program used for this purpose is given in Appendix III . Less commonly employed methods are those of planimetry and the relationship between the weight of paper

under the profile and the weight per unit area. The % bioavailability for oral absorption can then be calculated from equation (46):-

$$\% \text{ Bioavailability} = \frac{AUC_{po}}{AUC_{iv}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots \quad (46)$$

where AUC_{po} and AUC_{iv} are the areas under the respective oral and intravenous plasma level profiles, for the same dose. This equation may be used as a means of determining the changes in bioavailability associated with variations in particle size and formulation etc following the oral administration of SSA to man and the rat. Similarly, if a correlation can be shown to exist between drug plasma and saliva concentrations then the corresponding AUC values for saliva studies can be used, according to equation (47):-

$$\% \text{ Bioavailability} = \frac{AUC'_{SAL}}{AUC''_{SAL}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots \quad (47)$$

where AUC'_{SAL} and AUC''_{SAL} are the areas under saliva drug concentration vs time profiles for an experimental and reference formulation respectively.

3.2 HUMAN VOLUNTEER STUDIES

Physical and chemical studies on SSA have demonstrated that the drug is poorly soluble and exhibits dissolution behaviour that can be shown to be influenced by particle size. Also, formulatory manoeuvres, designed to enhance solubility and dissolution, have been carried out by the coprecipitation of SSA with polyvinylpyrrolidone (PVP), cholic acid (CA) and deoxycholic acid (DOCA) and examination of the crystal morphology of these coprecipitates has suggested that the observed alteration of the crystalline nature of the SSA:PVP coprecipitates might be reflected in the gastrointestinal absorption of the drug following its oral administration. Similarly the SSA:CA and SSA:DOCA coprecipitates may show in vivo behaviour exceeding that of the drug alone or drug:bile acid physical mixtures. This is especially true for the SSA:CA 1:1 molar ratio coprecipitates.

To examine the above possibilities it was decided to monitor the absorption of SSA from the gastrointestinal tract and to investigate the possible influences of experimental formulations on the bioavailability of the drug, in man, by measuring the appearance of salicylate in the saliva and urine. The first objective was, therefore, to establish that urinary excretion and saliva concentration data could provide an adequately discriminative and sensitive means of quantifying SSA absorption in man.

3.2.1 EXPERIMENTAL PROTOCOL

Human volunteers taking part in these studies were male, aged between 25 and 38 years (mean 27.5) with body weights ranging

from 64-93 kg (mean 82). All six subjects were instructed not to consume alcohol for 48 hours before SSA administration and throughout the entire experimental period. Subjects receiving any drug therapy involving the use of salicylates, or any subject who manifested any untoward effect on taking salicylates were omitted from these studies. In an attempt to provide between-subject standardisation of the gastrointestinal environment volunteers were instructed not to consume food after the normal evening meal on the day preceding any study. On completion of each experiment subjects reverted to their normal dietary habits.

CCLLECTION OF URINE. To promote an adequate flow of urine, water as customary beverages, was allowed ad libitum except that, on the day of each study, breakfast was to be no more than one cup of coffee or tea, and no further liquid was imbibed until four hours after dosing, excluding the volume of water required to facilitate swallowing of the dosage form (controlled at 200 ml). Urine was collected 30 minutes before drug administration, and at timed intervals thereafter, by direct micturition into a clean 250 ml measuring cylinder, 50 ml aliquots of the total measured volume collected at each time interval were retained for assay. Subjects were encouraged to void the bladder as completely as possible and to replace lost liquid by drinking an equal volume of water. All urine voided was collected for a total of 48 hours post-administration to ensure completion of salicylate excretion.

COLLECTION OF SALIVA. On the days of study subjects were asked not to use fluoride toothpastes which could be expected to interfere with the fluorimetric assay techniques. Prior to saliva collection the mouth was thoroughly rinsed with at least three changes of distilled water and emptied by swallowing. Mixed saliva was collected by free drainage from the lower lip into a clean McCartney bottle. Saliva flow was stimulated by applying varying degrees of pressure (not chewing) to a piece of Teflon (PTFE) held firmly between the premolar teeth. The saliva collected in this way was clear of cellular debris and entrapped air. In the studies designed to examine the correlation between plasma and saliva salicylate concentrations blood samples were removed at the midpoint of the saliva collection period, which was approximately 4-6 minutes. In all cases the volume of saliva produced was sufficient to allow removal of a 4 ml sample by pipette, this being stored at $-15^{o}C$ until assayed. In all studies the time at the midpoint of the collection period was used in analysis of the data. After taking any food or drink, collection of the next saliva sample was preceded by thorough rinsing of the buccal cavity with at least three changes of distilled water. Various drink substances expected to interfere with the salicylate fluorescence assay, eg tonic water, bitter lemon or other synthetic soft drinks and cordials, were avoided.

COLLECTION OF PLASMA. 2 ml samples of whole blood were removed by means of a syringe, via an indwelling cannula placed in the antecubital vein, and transferred to a heparinised centrifuge tube. The cannula was flushed with citrate anticoagulant after

the removal of each sample in order to prevent blockage. Plasma was obtained from the blood sample by centrifugation immediately after its collection and 0.5 ml aliquots were placed in screw-capped tubes to be stored at -15°C prior to assay. Pooled plasma used in the preparation of salicylic acid standards was obtained by centrifugation of heparinised blood taken by venupuncture from the antecubital vein of normal healthy volunteers.

3.2.2 ASSAY METHODS

MATERIALS. Commonly available organic solvents, reagents, buffer salts, etc, were used as supplied. Sørensen's phosphate buffer was prepared at pH 7.0 according to Documenta Geigy (1962). Hard gelatin capsules, size 0, were obtained from Parke-Davis and Co., Pontypool and heparin (5×10^3 units ml^{-1}) from the Boots Co, Nottingham.

FERRIC REAGENT consisted of 1.5 g ferric nitrate, $Fe(NO_3)_3.9H_2O$, dissolved in 31 ml of a 1 in 100 dilution of concentrated nitric acid and made up to 100 ml with distilled water. This stock reagent was kept in an amber glass bottle and diluted 1 in 20 prior to use.

GLASSWARE used in the fluorescence assays was cleaned by total immersion in a hot solution of Rapidex (Tool Importers Ltd, London), then three tap water rinses and overnight immersion in a dilute nitric acid solution, followed by three distilled water rinses and drying in a hot air oven.

INSTRUMENTATION. Visible spectrophotometric analysis of the salicylate-ferric ion purple chromophore was carried out using the Unicam SP1800 spectrophotometer and appropriate matched glass cuvettes.

Spectrophotofluorimetric analysis of the salicylate fluophore was achieved using the Aminco Bowman Spectrophotofluorimeter and the appropriate quartz cuvette.

AUTOCLAVING was carried out in a stainless steel autoclave (Taylor Rustless Fitting Co Ltd, Leeds).

3.2.2.1 Assay of Salicylate in Urine

Previous studies on the urinary excretion of salicylate after oral administration of SSA have shown that approximately 10% of the drug appears unchanged in the urine (Rubin, 1964; Hanzlik and Presho, 1925; MacDonald, 1973). Stability studies (See 2.9) and unpublished studies (MacDonald, 1973) have shown SSA to be readily hydrolysed under strongly alkaline conditions. This latter work demonstrated complete SSA breakdown to salicylic acid after autoclaving urine samples containing SSA with 0.1N sodium hydroxide for three hours, whereas the similar use of 12N sulphuric acid only produced an average of 15% hydrolysis in the same time period.

The method employed in this work relied on the production of a purple chromophore, between ferric ions and salicylic acid, and its subsequent measurement of visible spectrophotometry. Details of the analytical procedure are given as a flow diagram in

Fig. 3.2.1. Duplicate salicylic acid standard solutions (5-250 $\mu g.ml^{-1}$) prepared in pooled urine were analysed by the described method and the slopes of the lines (Fig. 3.2.2) were shown by a student 't' test not to be significantly different. The data were therefore subsequently pooled, submitted to linear least-squares regression analysis and the resultant equation was used to calculate the salicylate concentration of the experimental samples. The duplicate and pooled slopes are given in Table 3.2.1 with their appropriate standard errors.

	Slope $\pm$ SE ($\times 10^{-3}$)	Intercept $\pm$ SE ($\times 10^{-3}$	r	Student 't' test
A	5.344 ± 0.015	5.278 ± 1.865	0.999	$t_{obs} = 0.376$
B	5.337 ± 0.011	0.844 ± 1.311	0.999	$t_{tab} = 2.306$
Pooled	5.405 ± 0.086	0.993 ± 1.059	0.999	$n = 10$ $p' = 0.05$

Table 3.2.1 Student 't' Test Comparison Between Duplicate Standard Salicylic Acid Curves in Urine.

The total salicylate excreted for each study was calculated as the cumulative amount excreted, expressed as equivalents of SSA for each of the urine samples taken, and used to evaluate the % of the administered dose excreted.

3.2.2.2 Assay of Salicylate in Saliva

The method used was based on the production of a salicylate fluophore in phosphate buffer at pH 7.0 after ethereal extraction from acidified saliva and was an adaptation of that reported by Graham and Rowland (1972). The details of the assay procedure

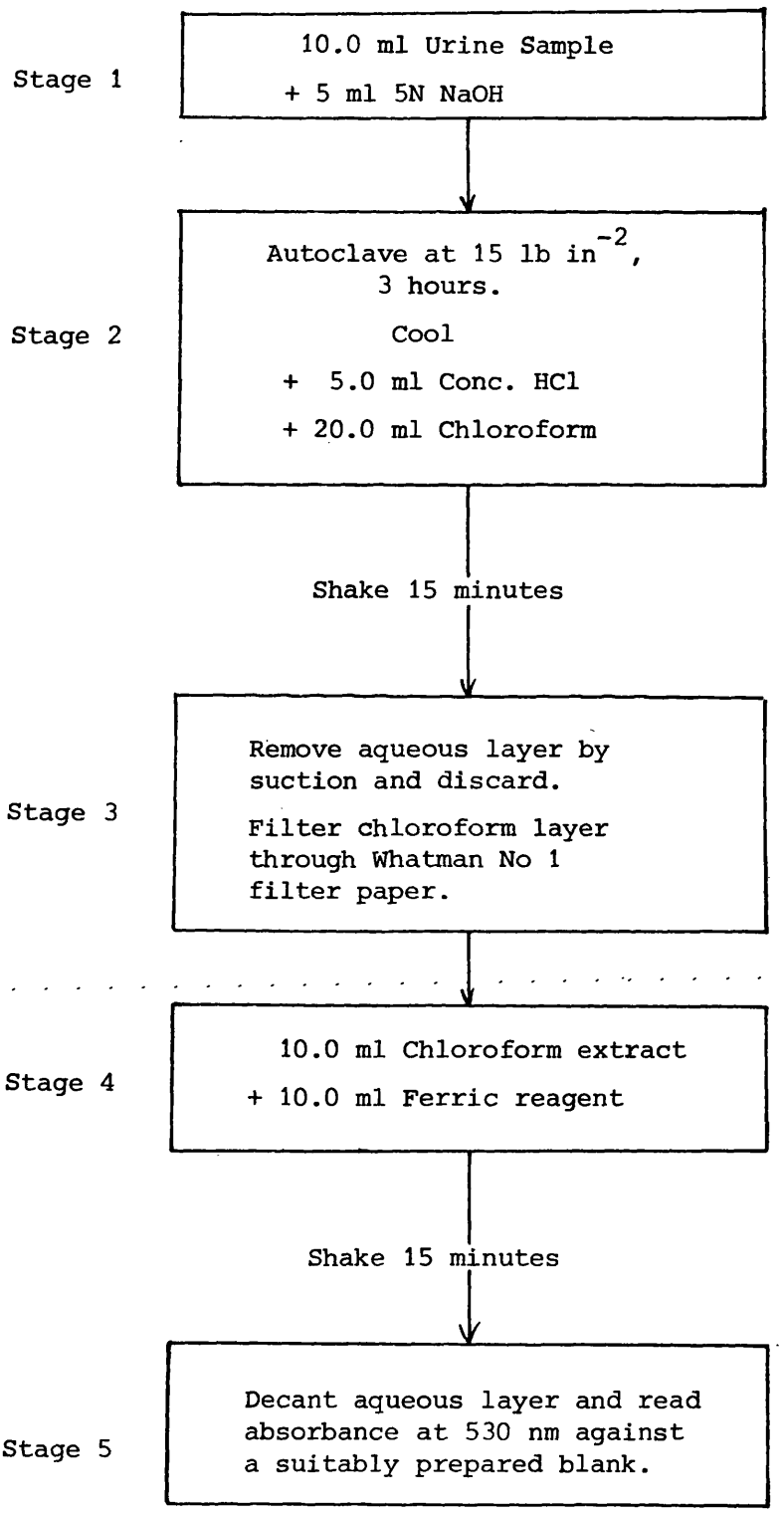


Fig. 3.2.1 Flow Diagram For The Assay Of Total Salicylate In Urine

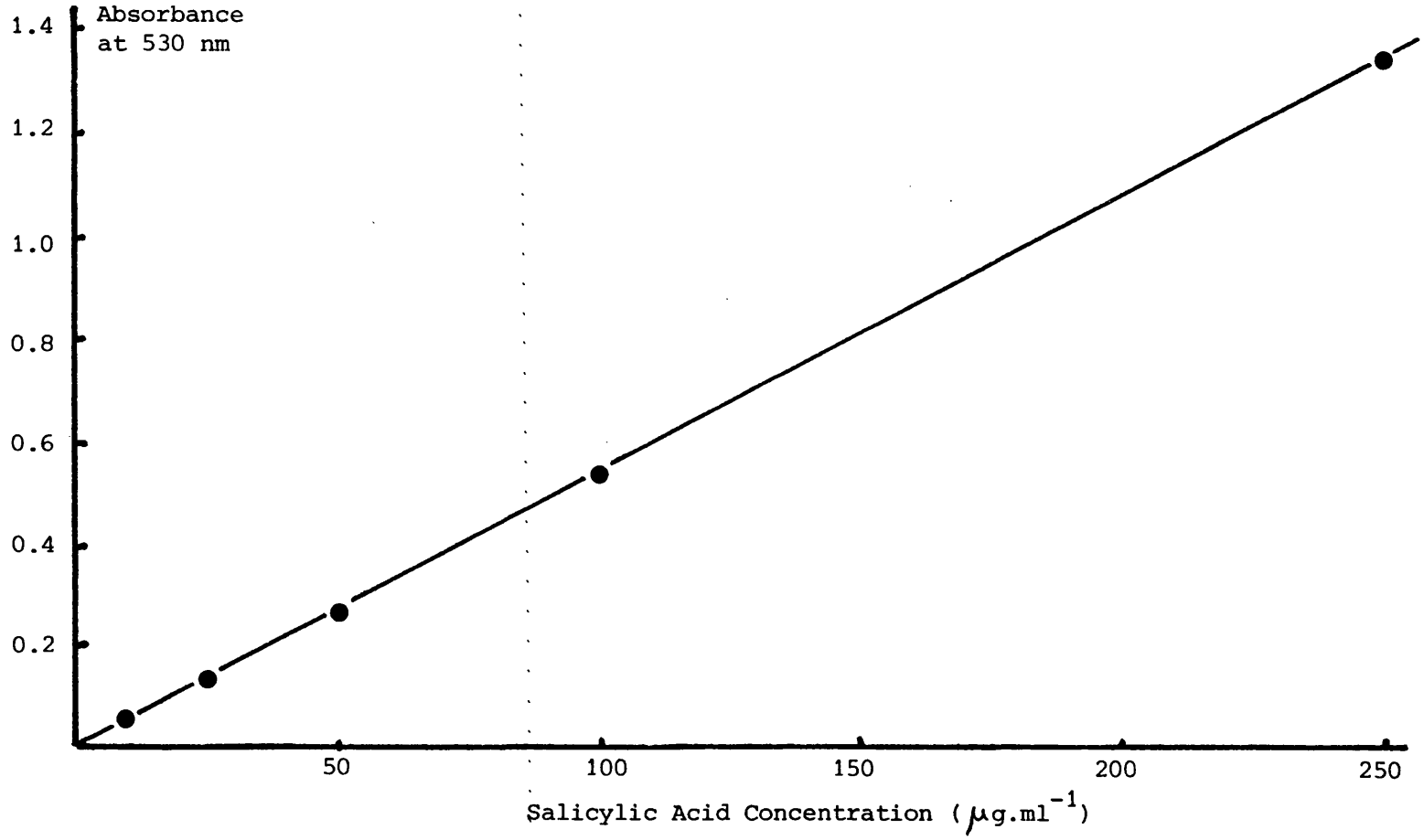


Fig. 3.2.2 Beer-Lambert Plot For Salicylic Acid Standards In Human Urine

are given as a flow diagram in Fig. 3.2.3. Plots of relative fluorescence vs concentration are shown in Fig. 3.2.4 for duplicate salicylic acid standards (0.05 - 3 $\mu g.ml^{-1}$) prepared in pooled saliva and assayed according to the method described. One of the standards was used to determine the activation and emission wavelength maxima used in subsequent analyses. These wavelength maxima were verified for each series of experimental samples particularly where formulatory adjuvants had been administered. Comparison of the two standard curves by a Student 't' test (Table 3.2.2) shows them not to be significantly different. The data were subsequently pooled and submitted to linear least-squares regression analysis, the equation from which was employed to calculate the salicylate content of the experimental samples.

	Slope $\pm$ SE	Intercept $\pm$ SE	r	't' test
A	488.2 $\pm$ 7.9	- 3.0 $\pm$ 11.7	0.999	t_{obs} = 0.319
B	491.4 $\pm$ 6.4	- 4.6 $\pm$ 9.4	0.999	t_{tab} = 2.101 N = 22 p′ = 0.05
Pooled	489.8 $\pm$ 4.9	- 3.8 $\pm$ 7.1	0.999	

Table 3.2.2 Student 't' Test Comparison Between Duplicate Salicylic Acid Standard Curves in Saliva

3.2.2.3 Assay Of Salicylate In Human Plasma

The method employed was essentially the same as that for saliva except that in Stage 1 (Fig. 3.2.3) 0.5 ml volumes of plasma were acidified with equal volumes of 4N HCl prior to ether extraction. Salicylic acid standards (1-50 $\mu g.ml^{-1}$) prepared in

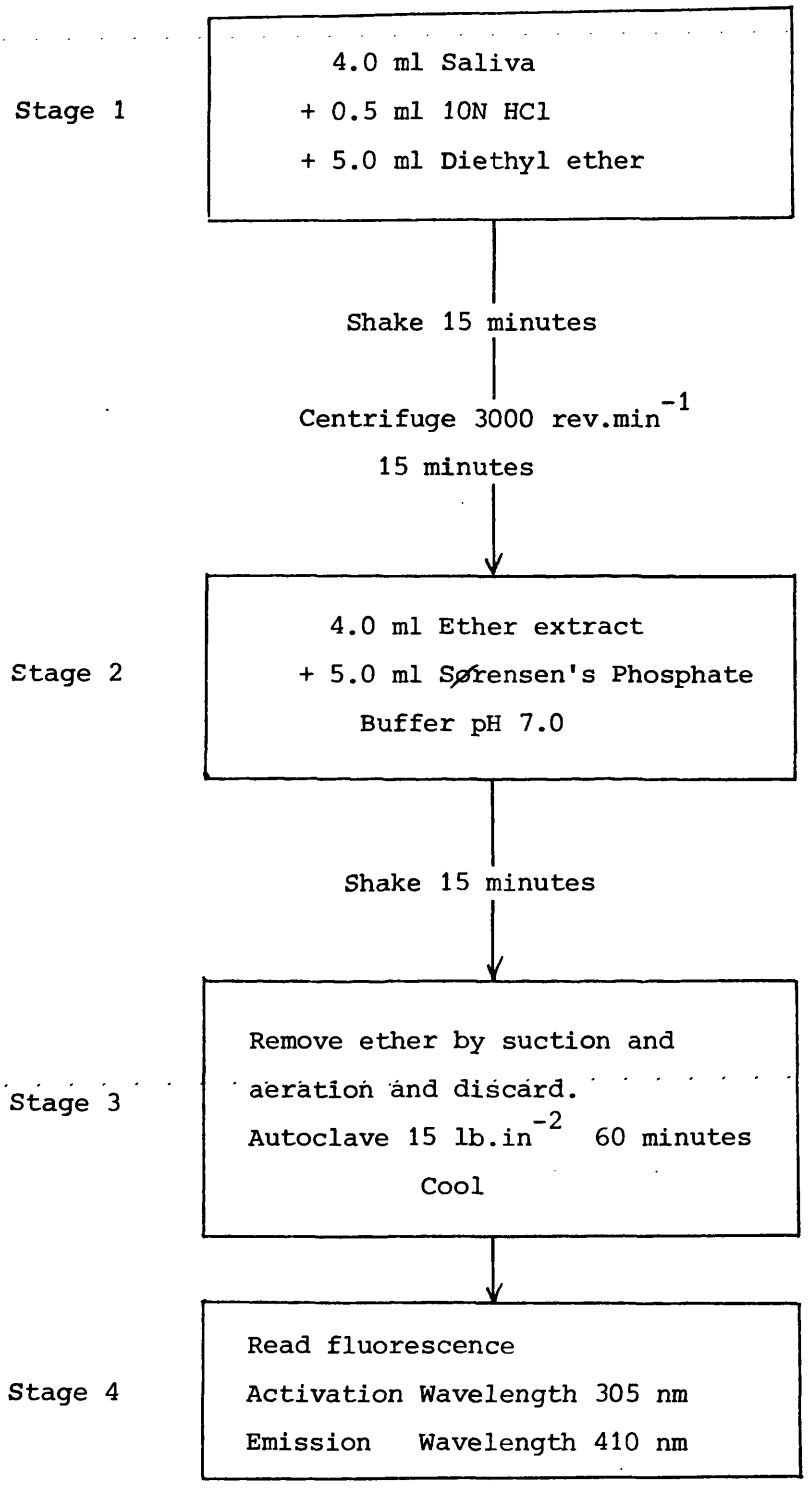


Fig. 3.2.3 Flow Diagram For The Assay Of Salicylate In Saliva

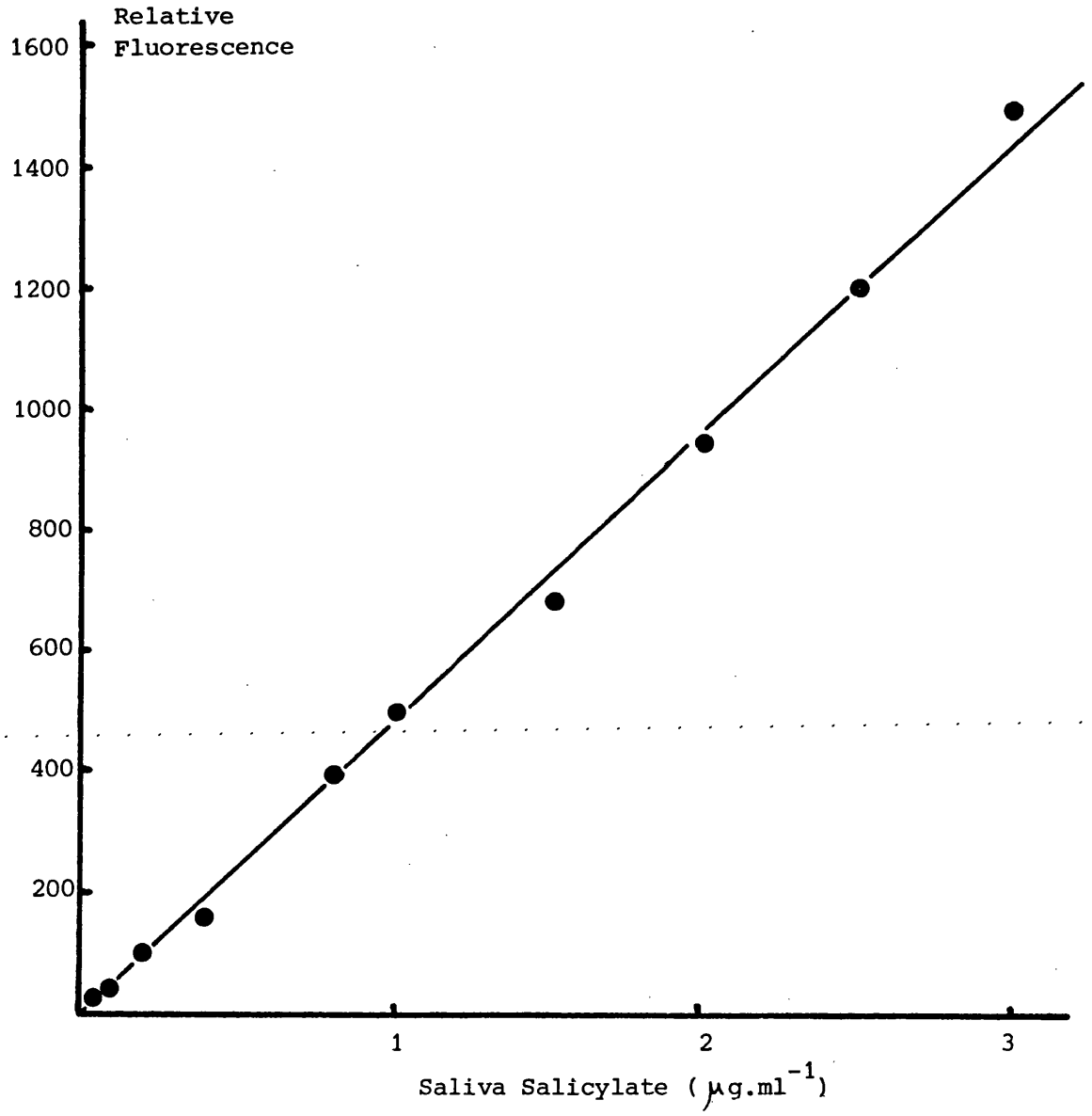


Fig. 3.2.4 Calibration Curve Of Fluorescence For Standard Solution Of Salicylic Acid In Human Saliva.

duplicate gave identical fluorescence wavelength maxima to those obtained for saliva standards and plots of relative fluorescence vs concentration (Fig. 3.2.5) were found, by a Student 't' test, not to be significantly different (Table 3.2.3). The data was therefore pooled and submitted to linear least-squares regression analysis to give the equation of the line used in subsequent calculation of the salicylate content of experimental samples. This equation accounted for the positive intercept of the standard curves, a value that was attributed to fluorescence derived from an unknown constituent of the plasma.

	Slope $\pm$ SE	Intercept $\pm$ SE	r	't' test
A	64.14 $\pm$ 1.03	53.35 $\pm$ 28.87	0.999	t_{obs} = 0.765
B	63.24 $\pm$ 0.58	58.79 $\pm$ 16.14	0.999	t_{tab} = 2.23 N = 14 p' = 0.05
Pooled	63.69 $\pm$ 0.57	56.67 $\pm$ 15.92	0.999	

Table 3.2.3 Student 't' Test Comparison Between Duplicate Salicylic Acid Standard Curves in Human Plasma.

Before proceeding with studies on SSA assurance of the validity of the experimental protocol was gained by investigating the saliva salicylate profile following oral administration of aspirin in solution (Solprin, Reckitt and Colman, Hull). Subjects received doses of either 300, 600 or 900 mg aspirin in solution in 200 ml of water, saliva samples were collected for a period of 28 hours and assayed for salicylate according to the method described. The resultant concentration-time profiles are given in Fig. 3.2.6 and in all cases rapid aspirin absorption is

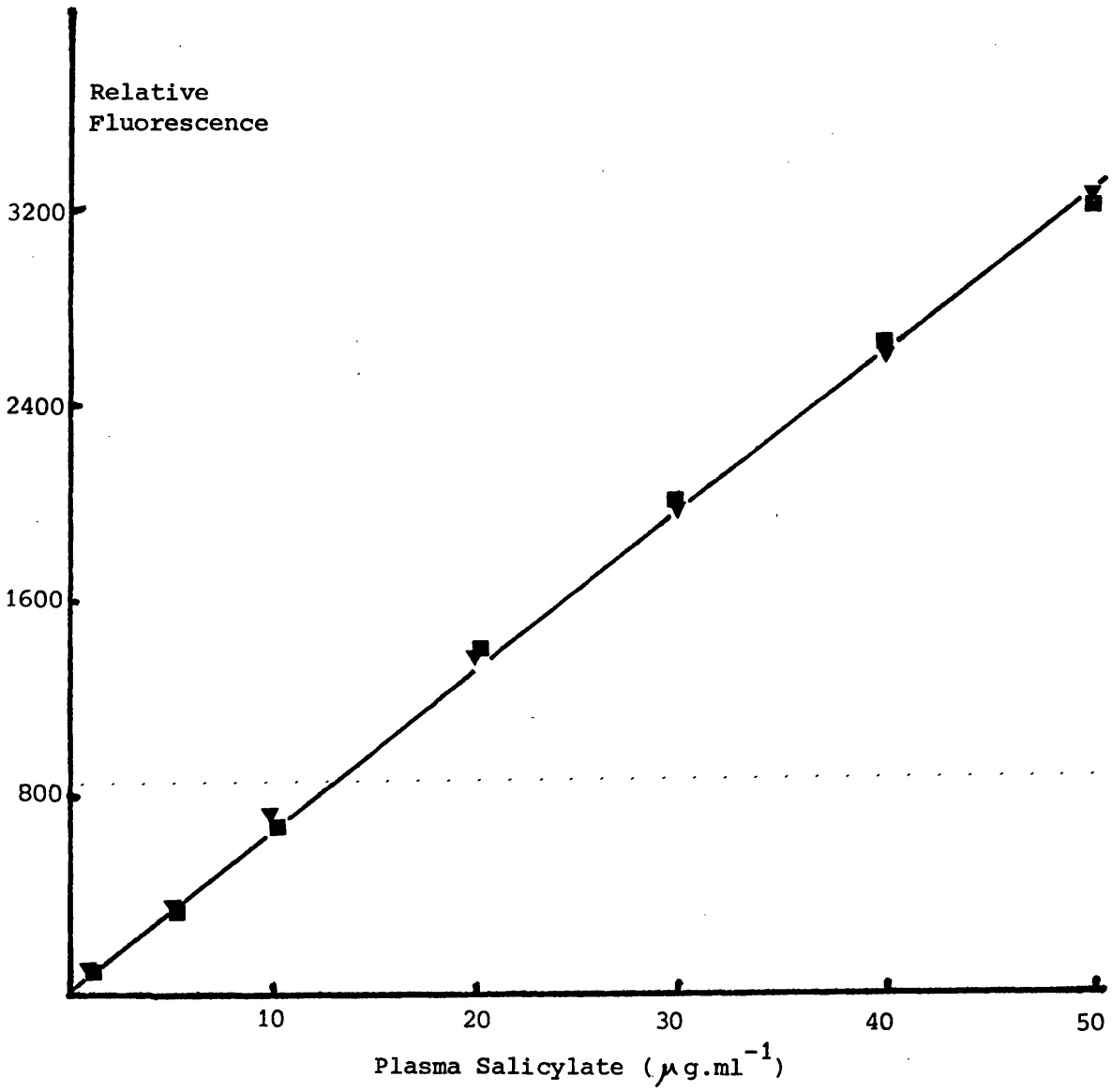


Fig. 3.2.5 Calibration Curve Of Fluorescence For Duplicate Standard Solutions Of Salicylic Acid In Human Plasma.

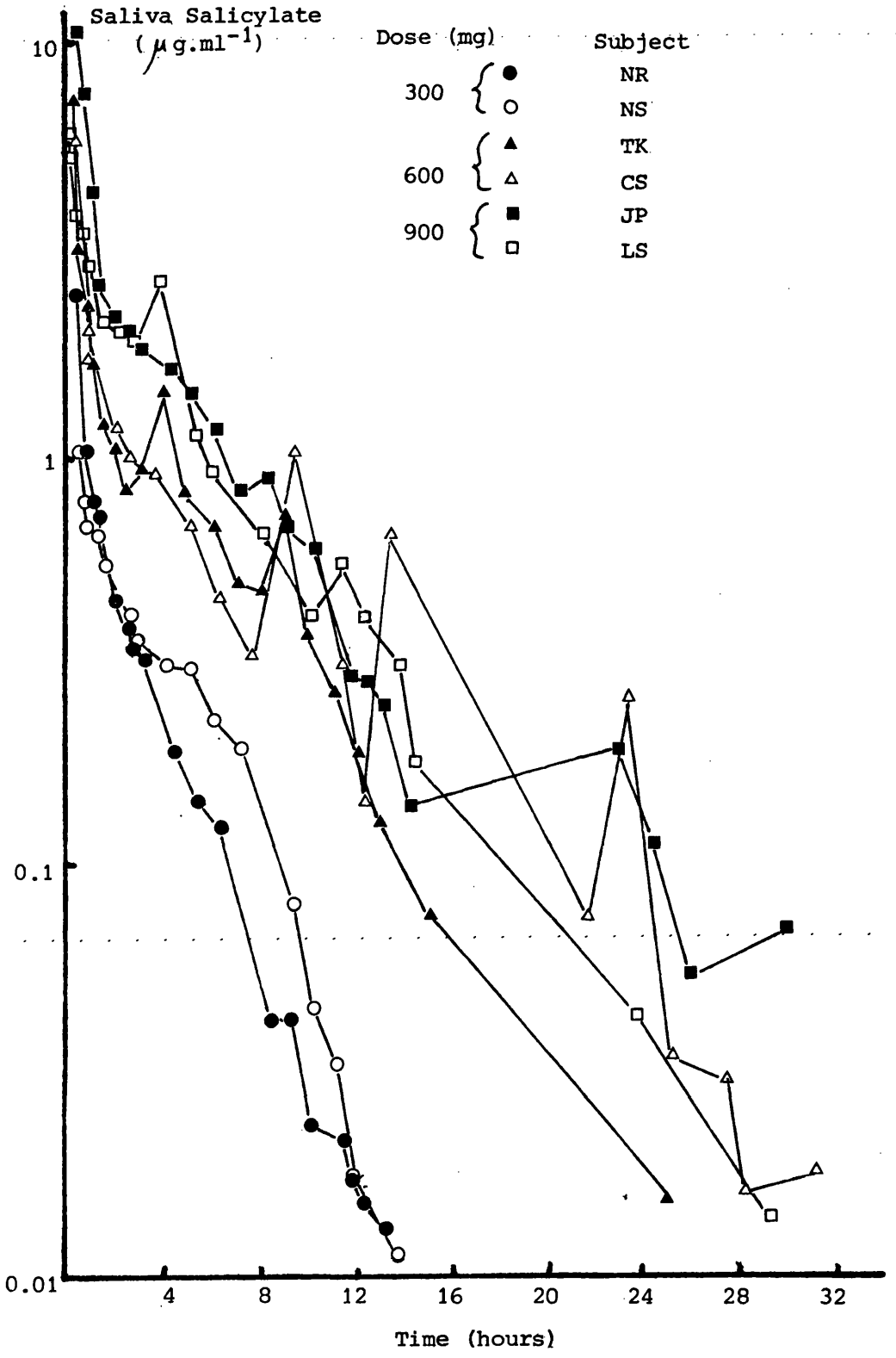


Fig. 3.2.6 Saliva Salicylate (log scale) vs Time Profiles For Aspirin Following Oral Administration In Solution At Three Dosage Levels.

indicated by the absence of an initial absorptive phase. One subject (CS) exhibited considerable scatter of salivary salicylate concentrations and the data could not be used in the calculation of an elimination rate constant from the terminal log-linear phase of the profile. For two other subjects (LS and TK) secondary and tertiary saliva peaks occurred at approximately four and nine hours after dosing which could be associated with the intake of food. Possible explanations for these mid-profile peaks will be discussed later. Subjective discrimination of the terminal data points and their subsequent linear least-squares regression analysis shows that the overall salicylate apparent first order elimination rate constants for each subject are comparable (Table 3.2.4). The data points used for these calculations were those visually distributed about a straight line that could be drawn through the terminal elimination phase of the profile. Further examination of the entire profile in this manner revealed a deviation from linearity for the 0 - 6 hour samples at doses of 600 and 900 mg.

Dose (mg)	300		600	900	
Subject	NR	NS	TK	JP	LS
k_{el} (hr^{-1})	0.3490	0.2585	0.2662	0.2536	0.1848
$\pm$ SE	0.0112	0.0230	0.0370	0.0217	0.0054
r	0.993	0.977	0.972	0.975	0.995
$t_{\frac{1}{2}}$ (mins)	119.2	160.8	156.2	164.0	225.0

Table 3.2.4 Apparent First Order Elimination Rate Constants and Half Life of Salicylate In Man After Oral Administration Of Aspirin In Solution.

The elimination half-lives determined are of the same order as those reported by Graham and Rowland (1972). The initial high salicylate concentrations are probably due to buccal contamination resulting from drug residing in the mouth following dosing with a solution, and would not, of course, be present had aspirin been given in capsules. However, the aim of the initial experiment was fulfilled and the assay in saliva and the general experimental protocol provide a valid method for monitoring salicylate absorption and elimination following oral administration of aspirin. The areas under the saliva salicylate time profiles were calculated for each subject by use of the trapezoidal rule and are given in Table 3.2.5.

Dose (mg)	Subject	AUC ($\mu g.ml^{-1}$ x min)
300	NS	193.49
	NR	175.51
600	TK	762.90
900	LS	1199.08
	JP	1557.58

Table 3.2.5 Values For Area Under Saliva Level Curve Following Oral Administration Of Aspirin In Solution

The values from Table 3.2.5 are also plotted in Fig. 3.2.7 and show a linear relationship between AUC and dose. The AUC value of 762.9 $\mu g.ml^{-1}$ x min calculated for subject TK, who received 600 mg aspirin, compares favourably with the values obtained for an oral dose of 650 mg aspirin obtained by Graham and Rowland (1972).

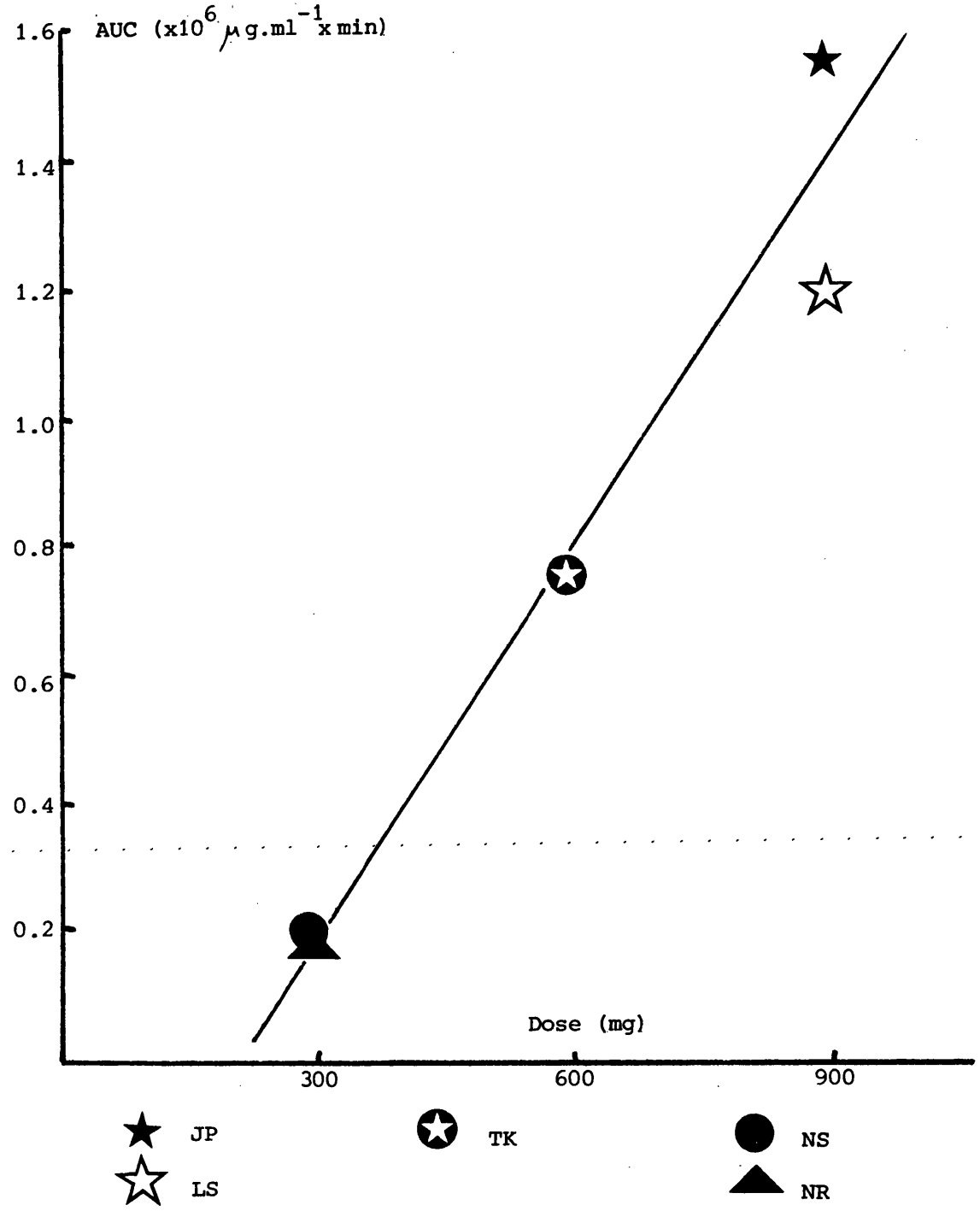


Fig. 3.2.7 Relationship Between The Area Under The Saliva Level Curve (AUC) And Dose Following The Oral Administration Of Aspirin In Solution.

The proportionally higher figure quoted for TK is probably the result of a combination of both initial buccal contamination and greater aspirin bioavailability from solution, although no firm conclusions can be drawn. Similarly, the negative intercept of the AUC vs dose plot may be due to exaggerated AUC values resulting from buccal contamination the degree of which would be significantly influenced by the dose of aspirin given.

Preliminary urinary excretion studies were then performed to establish the ability of urinary excretion data to differentiate between the in vivo behaviour of SSA when given orally, as a solution or as encapsulated material. The poor solubility of SSA in 0.1N HCl at 37° (80 μg.ml^{-1}) indicated that, if given in solution as the soluble salt then, depending on the dose, precipitation may occur in the acidic environment of the stomach and any incurred unorthodox absorption may therefore be reflected by the urinary excretion of total salicylate.

3.2.3 URINARY EXCRETION OF SALICYLATE AFTER ORAL ADMINISTRATION OF SSA IN SOLUTION AND CAPSULES

Using a crossover design, with a week between studies, six subjects received 60 mg SSA either as a solution or as 150-180 μm particle size material in a single hard gelatin capsule. The solution was prepared as for the recrystallisation studies (See 2.10) and both doses were taken with 200 ml of water. Urine samples, collected at zero time, and at appropriate time intervals for 48 hours, were assayed for total salicylate as described. Urinary salicylate excretion is represented in Fig. 3.2.8 as plots of the log % unexcreted vs time according to the 'Sigma-

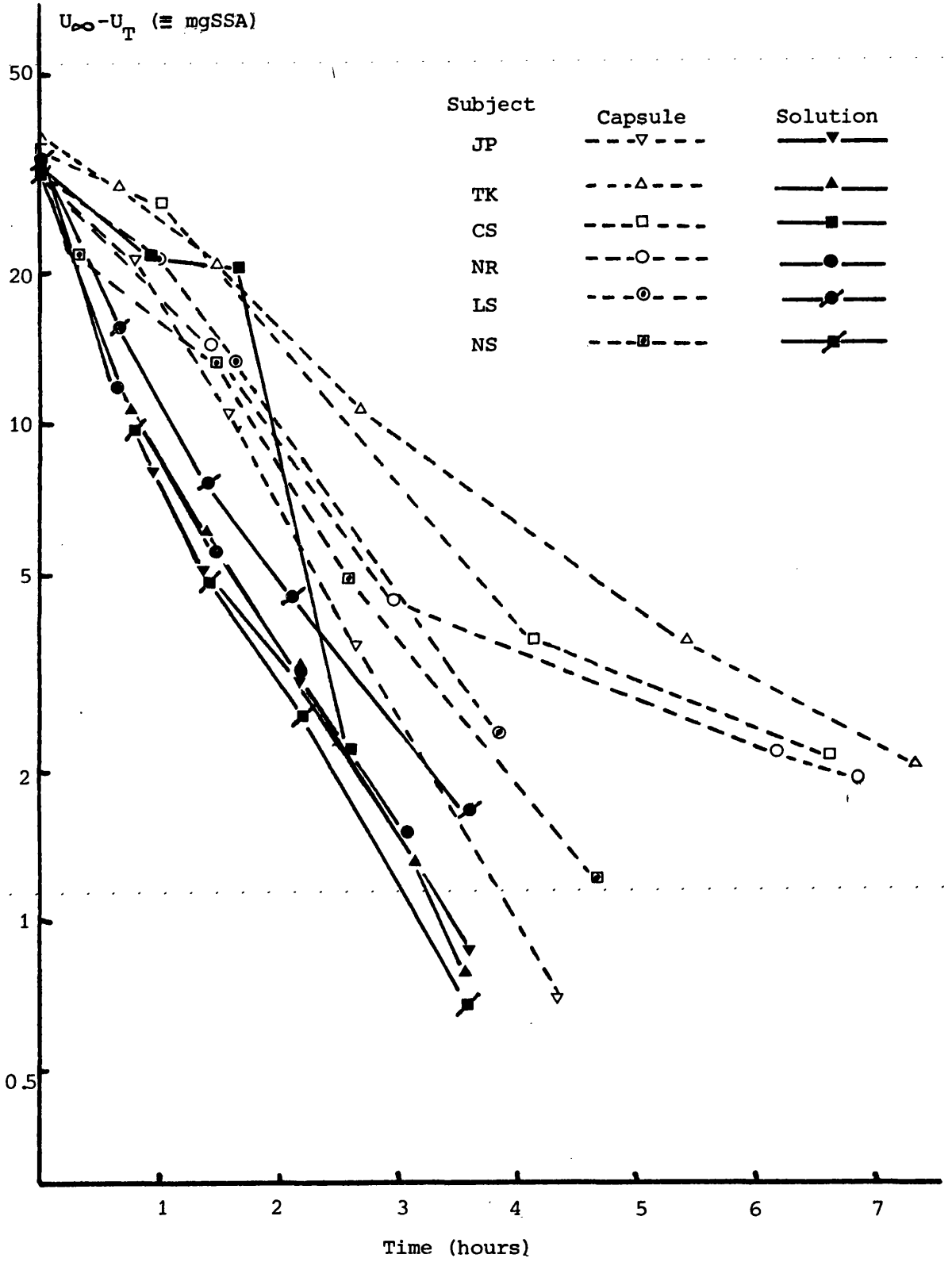


Fig. 3.2.8 Sigma-minus Plots Obtained Following Oral Administration Of SSA In Solution (closed symbols) And In Capsules, 150-180 μm (open symbols). Dose 60 mg.

minus' method described earlier (See 3.1 equation (45)). These plots show that the excretion for the two dosage forms follow different profiles and that the elimination for the solution exhibits less inter-subject variation than that for the capsule. This may be explained by possible variations in SSA absorption associated with erratic and prolonged dissolution of the poorly soluble particulate material under in vivo conditions. It is also evident that if in vivo precipitation had occurred to any significant extent then the log % unexcreted vs time profiles for the solution would have shown a greater degree of inter-subject variation than that observed. However, the lack of significant difference between the % dose excreted for either formulation (t_{obs} = 0.825; t_{tab} = 2.228; N = 12, p' = 0.05) is indicative of precipitation occurring since complete absorption from solution would have been anticipated. The apparent first order elimination rate constants and respective half-lives, calculated by regression analysis of the log-linear terminal section of the profiles are given in Table 3.2.6. It is evident from the k_{el} values given that their variability is greater, following oral administration of encapsulated material, than that for solution. These observations will be further discussed with respect to the recrystallisation studies previously reported (See 2.10).

The ability of urinary excretion data to discriminate between the absorption of the above formulations is somewhat limited and it was considered unable to provide an adequate means of differentiating between particle size and the influences of coprecipitation on SSA absorption. Studies were therefore

		LS	JP	NR	CS	NS	TK	Mean ± SE
First Order Elimination Rate Constant ± SE ($x10^{-1}hr^{-1}$)	SOLUTION	4.095 ± 0.27	4.944 ± 0.16	5.270 ± 0.64	ND*	5.117 ± 0.30	4.737 ± 0.39	4.833 ± 0.205
	CAPSULE	3.950 ± 0.08	4.915 ± 0.13	ND	ND	3.810 ± 0.32	2.033 ± 0.08	3.677 ± 0.601
Elimination Half Life (mins)	SOLUTION	101.5	84.10	78.9	ND	81.3	87.8	86.92 ± 3.95
	CAPSULE	105.3	84.60	ND	ND	109.1	204.5	125.88 ± 26.76
Extent Dose Excreted (%)	SOLUTION	57.1	53.7	57.4	54.0	51.3	56.9	55.07 ± 1.0
	CAPSULE	53.1	56.7	53.9	63.9	50.4	66.0	57.33 ± 2.6

* ND Not Determinable

Table 3.2.6 Elimination and Excretion Data for The Urinary Elimination of Salicylate Following Oral Administration of SSA (60 mg) in Solution and Capsules

performed to establish the ability of salivary salicylate levels to provide a non-invasive method for the assessment of the disposition of salicylate following oral administration of SSA.

3.2.4 CORRELATION OF SALIVA WITH PLASMA SALICYLATE CONCENTRATION FOLLOWING ORAL ADMINISTRATION OF SSA IN AN AQUEOUS SUSPENSION.

Six volunteers received either 300, 600, 900 mg of 'micronised' SSA as an aqueous suspension in 100 ml distilled water; a further 100 ml, used to rinse the mouth, was also swallowed. Plasma and saliva samples were taken at zero time and at time intervals until seven hours after dosing. Each plasma sample was obtained by centrifugation of heparinised blood removed at the mid-point of the saliva collection period. The resultant plasma and saliva salicylate concentration vs time profiles are shown in Figs. 3.2.9 and 3.2.10 respectively for each of the three dosage levels. From these profiles the approximate peak salicylate times and concentrations were obtained and are given in Table 3.2.7.

		Plasma Peak		Saliva Peak	
Dose (mg)	Subject	time (hr)	Concentration ($\mu g.ml^{-1}$)	time (hr)	Concentration ($\mu g.ml^{-1}$)
300	BM	2.0	14.0	2.0-4.0	0.48
	SM	2.0	12.0	ND*	ND
600	CS	1.0-2.0	23.0-27.0	4.0	0.95
	NR	2.0	36.0	2.0-4.0	1.13-1.50
900	JP	3.0	55.0	4.0	3.7
	LS	1.5-3.0	50.0-55.0	1.5-3.0	2.4-2.8

* ND Not Determinable

Table 3.2.7 Approximate Peak Plasma and Saliva Times and Concentrations of Salicylate Following Oral Administration of SSA In Aqueous Suspension.

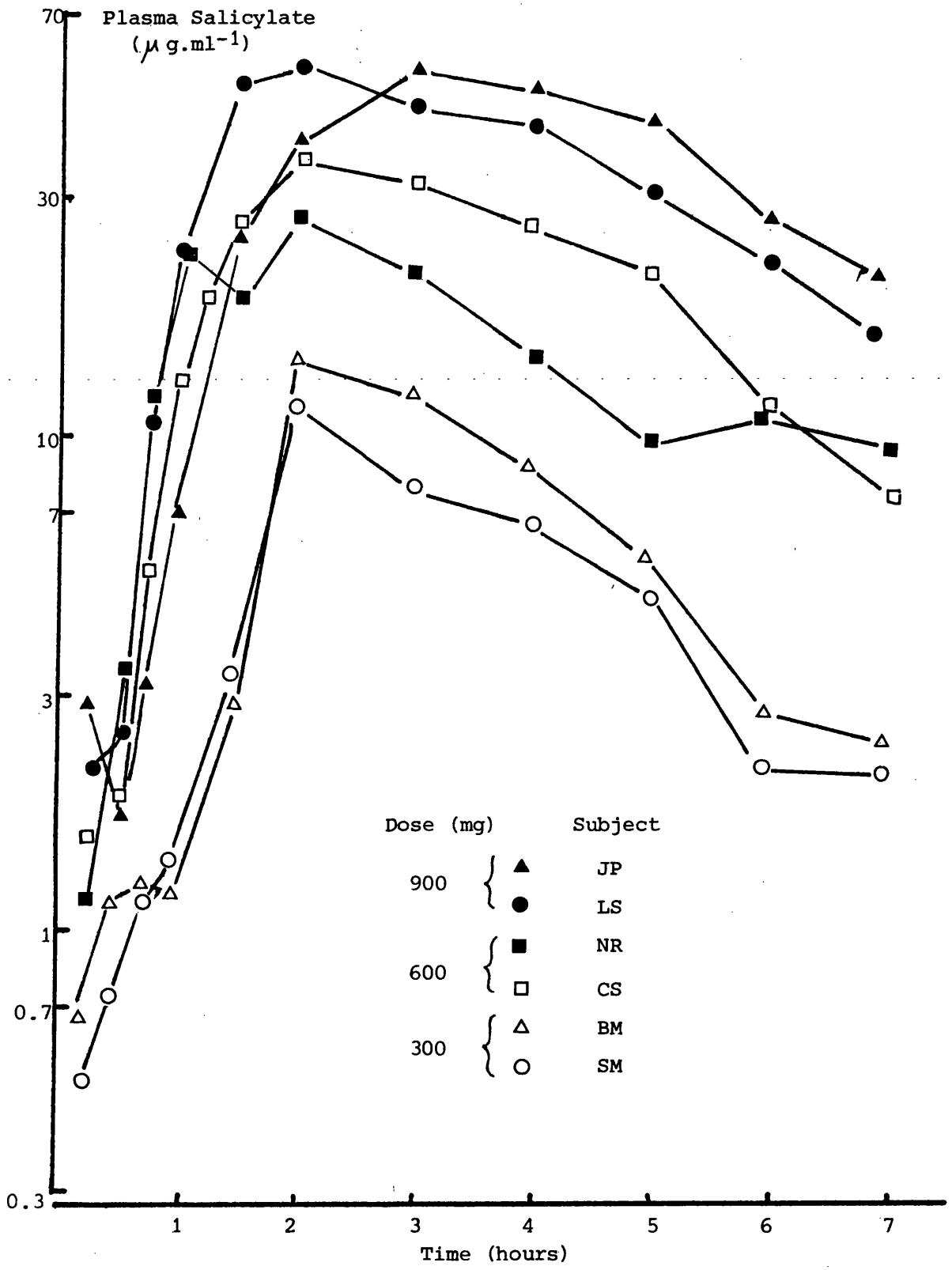


Fig. 3.2.9 Plasma Salicylate (log scale) vs Time Profiles For SSA Following Oral Administration In Suspension At Three Dosage Levels.

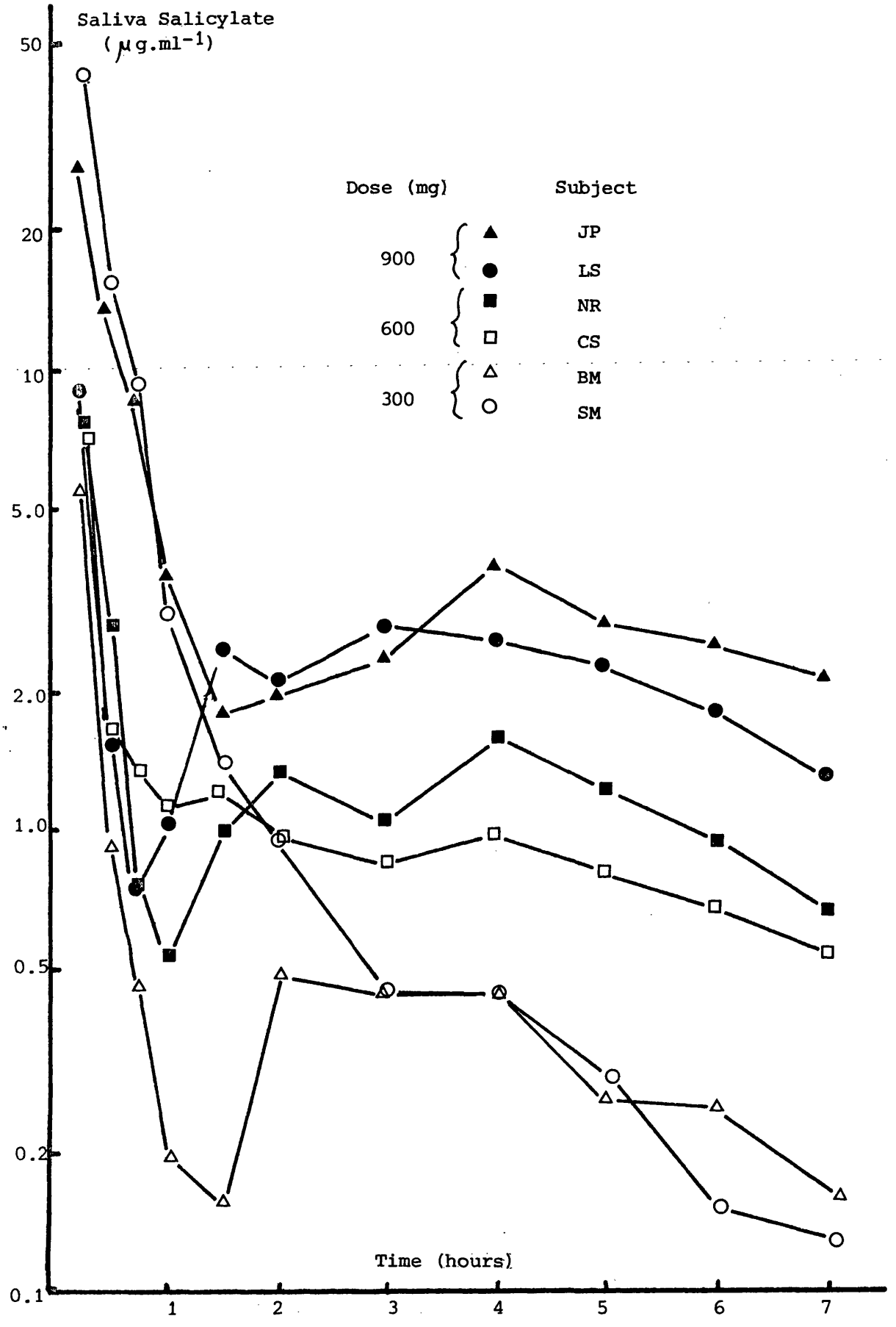


Fig. 3.2.10 Saliva Salicylate (log scale) vs Time Profiles For SSA Following Oral Administration In Suspension At Three Dosage Levels.

The correlation of saliva with plasma salicylate concentration is shown in Fig. 3.2.11 as a plot of saliva vs plasma concentration for all samples taken after two hours post administration. Earlier samples are not included due to the high degree of contamination of the saliva with residual drug in the buccal cavity. The amount of extraneous SSA in the mouth was sufficient in all cases to mask the majority of the absorptive phase of the saliva. Fig. 3.2.11 clearly shows that the saliva-plasma correlation changes with time and only stabilised after six hours when both plasma and saliva profiles entered the elimination phase. The mean ratio of salicylate concentration in the saliva to that in the plasma is given for each subject in Table 3.2.8. The values used in calculation of the mean exclude those exhibiting obvious salicylate contamination of the pre-two hour saliva samples. The coefficients of variation associated with these saliva/plasma values vary from 16-37% even though the mean ratios for the individual subjects are of the same order of magnitude, ranging from 0.051-0.067.

Dose SSA (mg)	Subject	Mean Saliva Plasma Ratio	Standard Deviation	Coefficient of Variation (%)
300	BM	0.0620	0.0103	16.61
	SM	0.0516	0.0177	34.30
600	CS	0.0586	0.0148	25.30
	NR	0.0510	0.0188	36.86
900	JP	0.0604	0.0161	26.66
	LS	0.0667	0.0182	27.28

Table 3.2.8 Saliva/Plasma Salicylate Concentration Ratios Following Oral Administration of SSA in Aqueous Suspension.

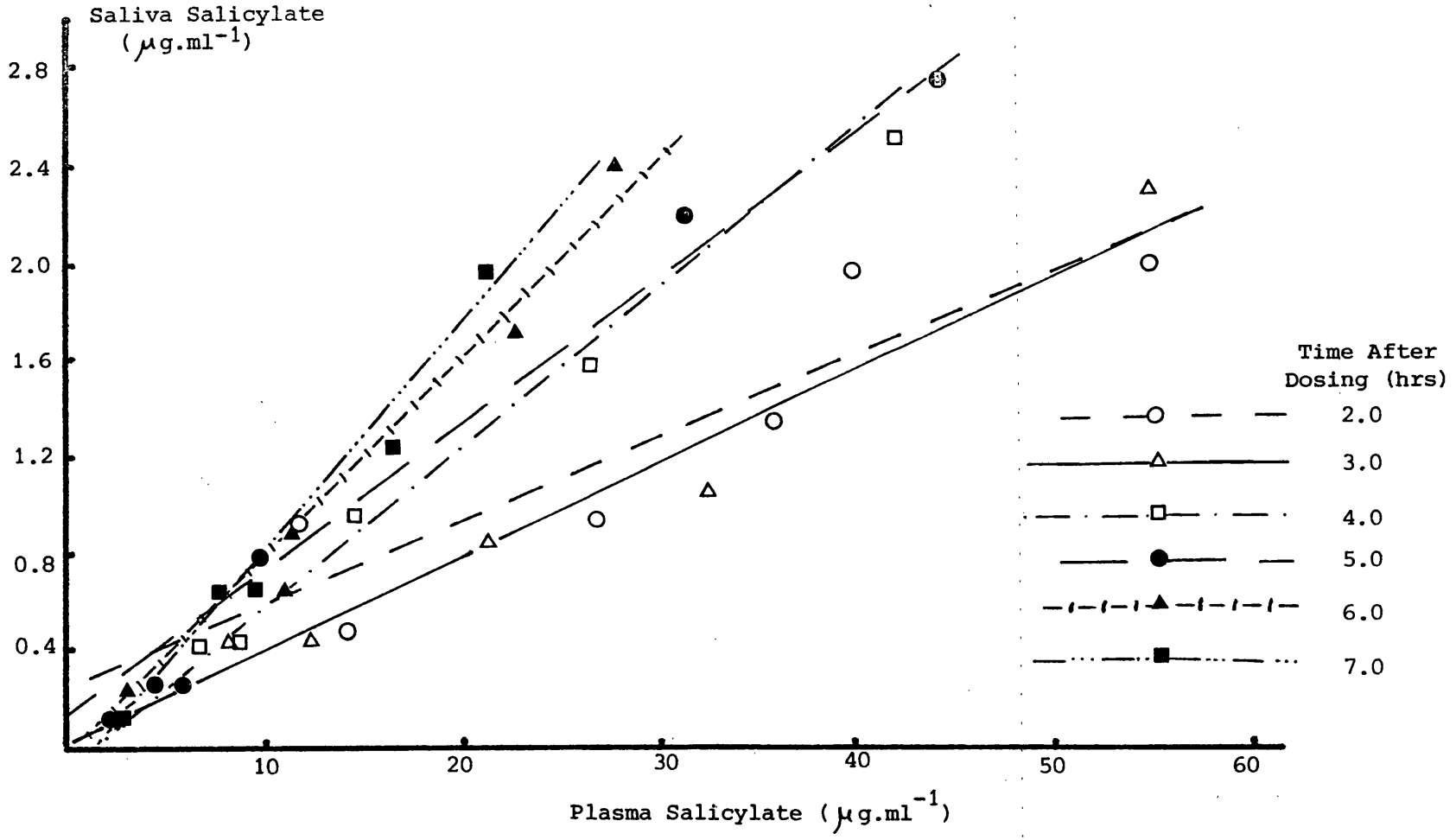


Fig. 3.2.11 Saliva/Plasma Salicylate Correlation With Respect To Time After Oral Administration Of SSA At Three Dosage Levels.

Change in the saliva/plasma salicylate ratio with time is also shown in Fig. 3.2.12. Some of the early (pre-two hour) samples are included to illustrate the erroneously high ratio associated with buccal contamination.

The most significant observations of this study are the saliva contamination resulting from oral administration of suspended material and the change in the plasma-saliva salicylate ratio with time. In an attempt to overcome the first of these disadvantages a second study was performed where SSA was given by mouth in hard gelatin capsules. From such a study it was anticipated that a complete saliva-plasma correlation could be achieved which may aid in the elucidation of the causative factors of the time-related change in that ratio.

3.2.5 CORRELATION OF SALIVA WITH PLASMA SALICYLATE CONCENTRATION FOLLOWING ORAL ADMINISTRATION OF SSA IN HARD GELATIN CAPSULES

The experimental protocol was identical to that of the previous study except that two subjects received 600 mg micronised SSA in two hard gelatin capsules with 200 ml of water. To aid characterisation of the respective peaks saliva and plasma samples were obtained at half-hourly intervals. The plasma and saliva salicylate profiles shown in Fig. 3.2.13 follow the same time course but the early saliva samples do not exhibit the contamination observed in the previous study. The peak plasma salicylate concentrations occurred at 1.5 and 3.0 hours for NS and LS respectively whereas both the corresponding peak saliva salicylate concentrations were approximately three hours post-administration. The delay in the occurrence of the saliva peak with respect to

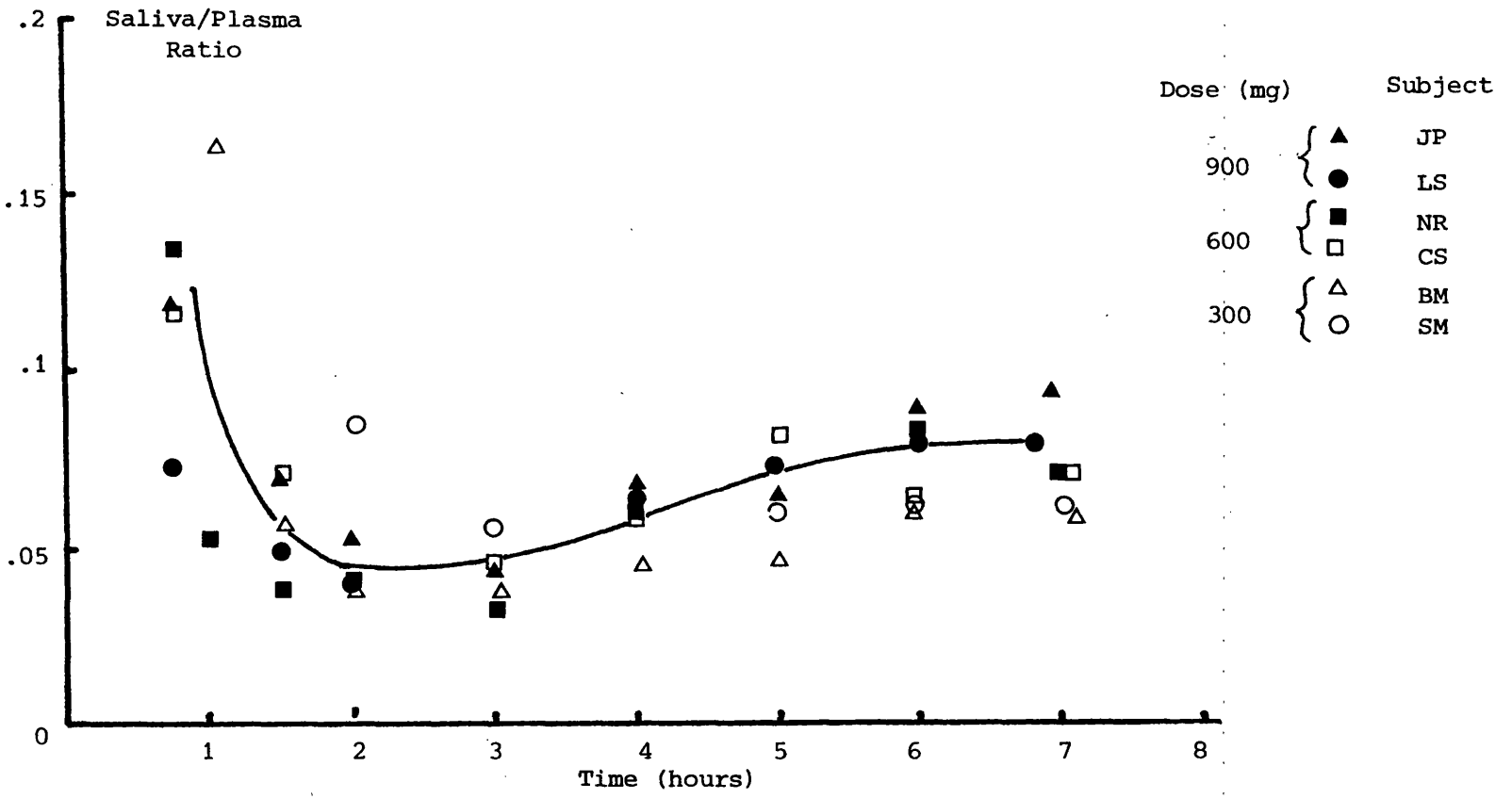


Fig. 3.2.12 Relationship Between Saliva/Plasma Concentration Ratio And Time Following Oral Administration Of SSA (micronised) At Three Dosage Levels.

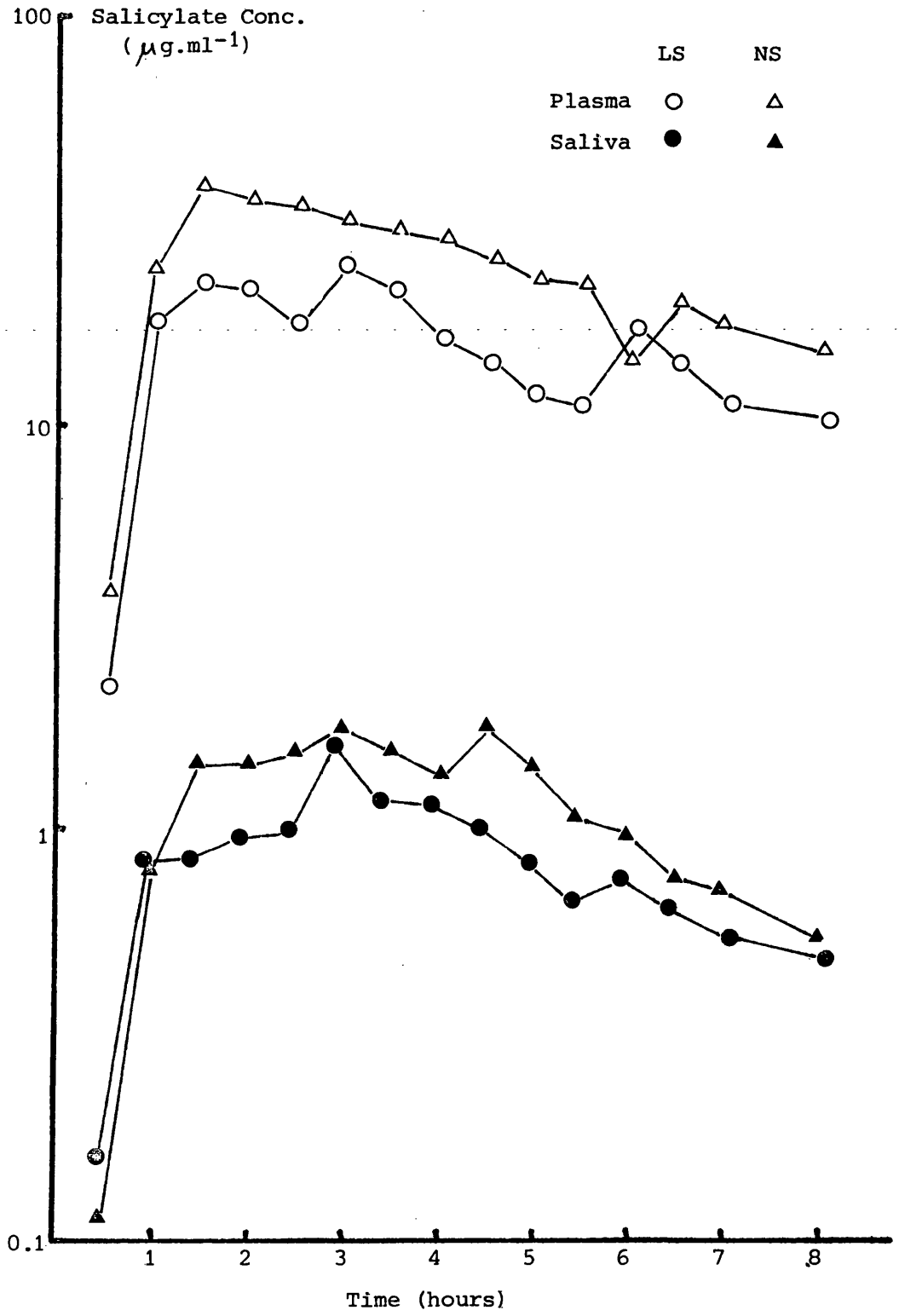


Fig. 3.12.13 Saliva (closed symbols) And Plasma (open symbols) Salicylate (log scale) vs Time Profiles Following Oral Administration In Hard Gelatin Capsules. Dose 600 mg.

that for plasma would explain the changes in the salicylate saliva-plasma ratio with time depicted in Fig. 3.2.14. This change in ratio is also represented by the low correlation coefficients for the plot of salicylate concentration in the saliva vs that in the plasma shown in Fig. 3.2.15. The data for the two subjects and the pooled data was submitted to linear least-squares regression analysis and the resultant correlation coefficients are given in Table 3.2.9.

	Individual		Pooled	
Subjects	Slope $\pm$ SE	r	Slope $\pm$ SE	r
NS	0.0423 ± 0.009	0.809	0.0423 ± 0.005	0.834
LS	0.0501 ± 0.009	0.833		

Table 3.2.9 Correlation Between Saliva and Plasma Salicylate Concentration Following Oral Administration of SSA in Capsules.

Apparent first order elimination rate constants were obtained from least-squares regression analysis of the appropriate log-linear sections of each of the plasma and saliva profiles and are given in Table 3.2.10 with a Student 't' test comparison.

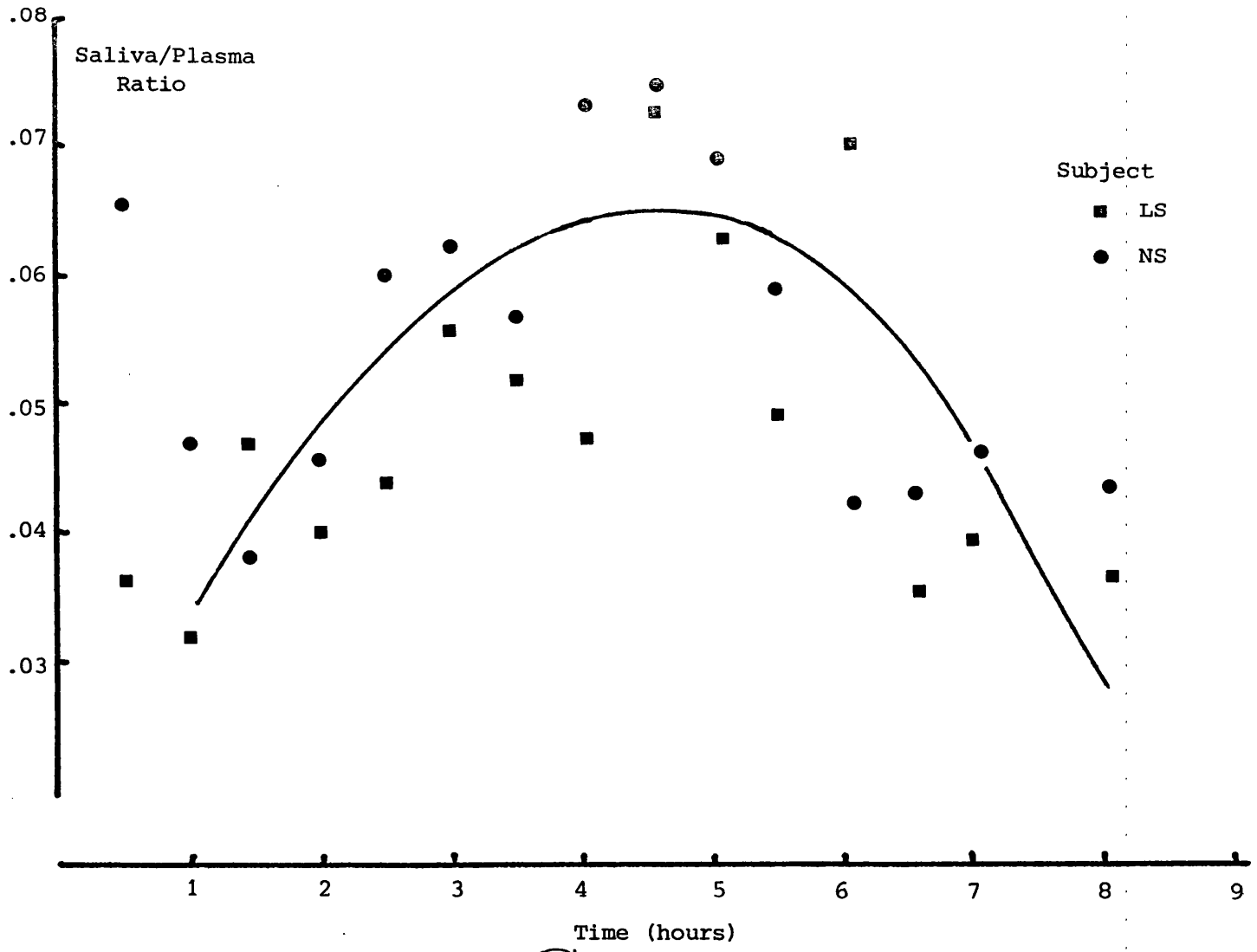


Fig. 3.2.14 Relationship Between The Plasma/Saliva Salicylate Concentration Ratio And Time Following Oral Administration Of SSA (75 - 105 μm) In Hard Gelatin Capsules. Dose 600 mg.

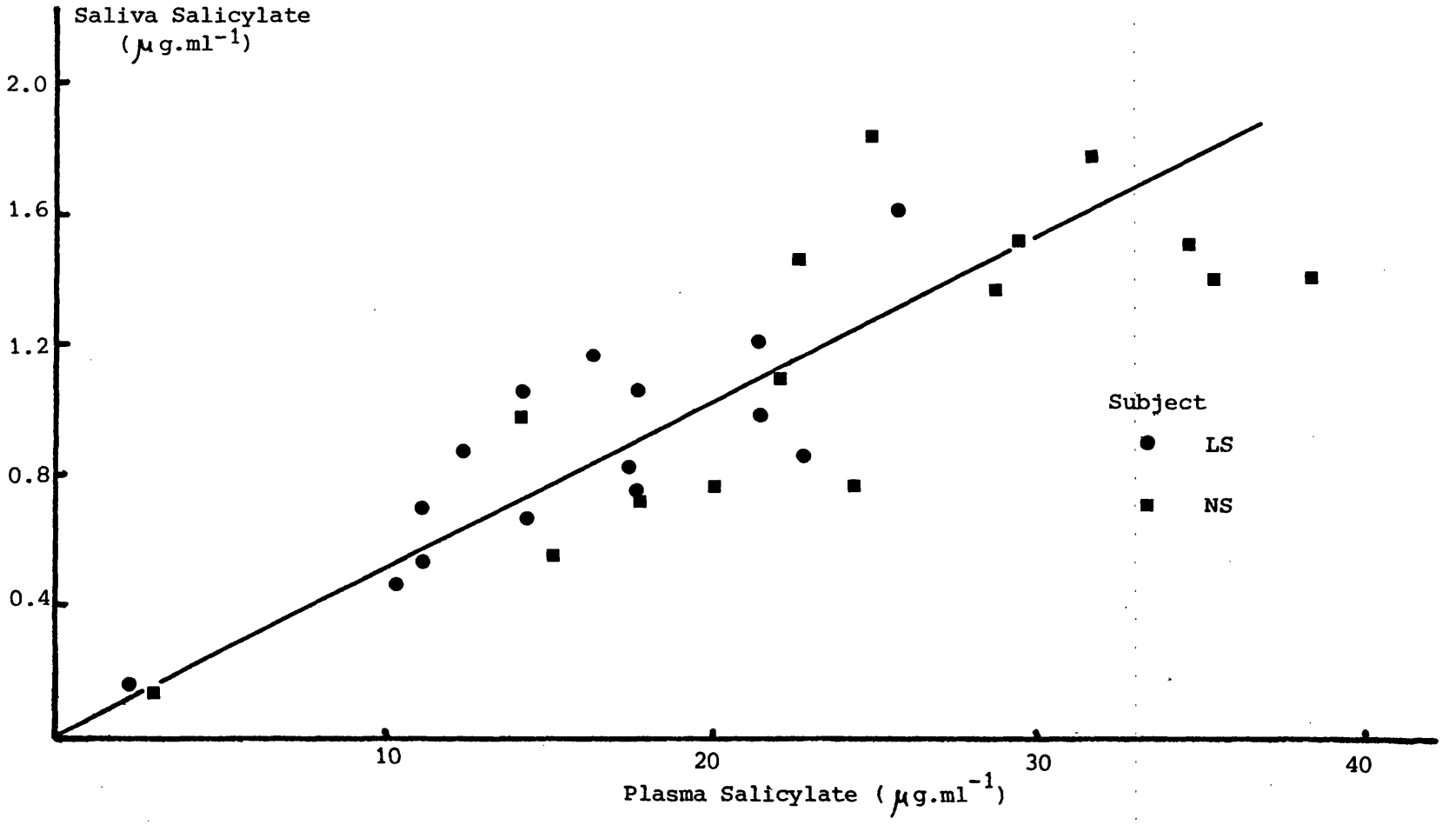


Fig. 3.2.15 Relationship Between Plasma And Saliva Salicylate Concentration Following Oral Administration Of SSA (75 - 105μm) In Hard Gelatin Capsules. Dose 600 mg.

Subject		Elimination Rate Constant $\pm$ SE (hr^{-1})	r	't' Test for Saliva	't' Test for Plasma
NS	SALIVA	0.2278 ± 0.022	0.969	$t_{obs} = 0.705$	$t_{obs} = 0.782$
	PLASMA	0.1399 ± 0.004	0.996	$n = 19$	$n = 25$
LS	SALIVA	0.2474 ± 0.017	0.982	$p' = 0.05$	$p' = 0.05$
	PLASMA	0.1201 ± 0.025	0.828	$t_{tab} = 2.132$	$t_{tab} = 2.080$

Table 3.2.10 Comparison of Overall Elimination Rate Constants Obtained From Salivary and Plasma Concentration Time Profiles Following Oral Administration of SSA in Capsules.

For both subjects the apparent first order elimination rate constants calculated from either plasma or saliva data are comparable since the Student 't' test shows them not to be significantly different. However, comparison of the elimination rate constants calculated from plasma and saliva data for each subject show, in both cases, that there is a significant different between the two. For NS, $t_{obs} = 3.931$; $N = 21$; $p' = 0.05$; $t_{tab} = 2.132$: For LS, $t_{obs} = 4.211$; $N = 23$; $p' = 0.05$; $t_{tab} = 2.093$. It can therefore be concluded that although saliva and plasma salicylate profiles can be used in their own right, to describe the time course of SSA absorption and salicylate elimination, it has been demonstrated that there is no direct relationship between the two in the subjects used. Saliva levels cannot therefore be used to describe the sequence of pharmacokinetic events occurring in the plasma.

Since drug elimination can be studied by the rate and extent of the appearance of unchanged drug and/or drug metabolites in the urine it was of interest to establish any degree of similarlity between elimination rate constants calculated from urinary excretion data with those derived from saliva data.

3.2.6 SALIVA CONCENTRATION VS TIME PROFILE AND URINARY EXCRETION OF SALICYLATE FOLLOWING ORAL ADMINISTRATION OF SSA IN HARD GELATIN CAPSULES

Five subjects received 600 mg micronised SSA in two hard gelatin capsules with 200 ml water. Saliva and urine samples were taken at zero time and at appropriate times thereafter, the resultant saliva salicylate concentration vs time profiles and 'Sigma-minus' plots are shown, for each subject, in Figs. 3.2.16 and 3.2.17 respectively. The corresponding apparent first order elimination rate constants are given in Table 3.2.11. Comparison of these rate constants by a Bartlett Test (See Appendix I) shows them to come from the same distribution ($\chi^2_{obs} = 1.671$; $p' = 0.05$; $N = 70$; $\chi^2_{tab} = 90.53$) and not to be significantly different.

Comparison between the apparent first order urinary elimination rate constants for this study with those where the SSA dose was 60 mg shows them to be significantly different $t_{obs} = 2.682$; $N = 9$; $p' = 0.05$; $t_{tab} = 2.365$. A possible explanation for this is derived by examination of the accompanying salicylate saliva vs time profiles obtained at the higher 600 mg dose (Fig. 3.2.17). From these profiles a second absorptive phase is indicated by the maintenance of a constant salicylate concentra-

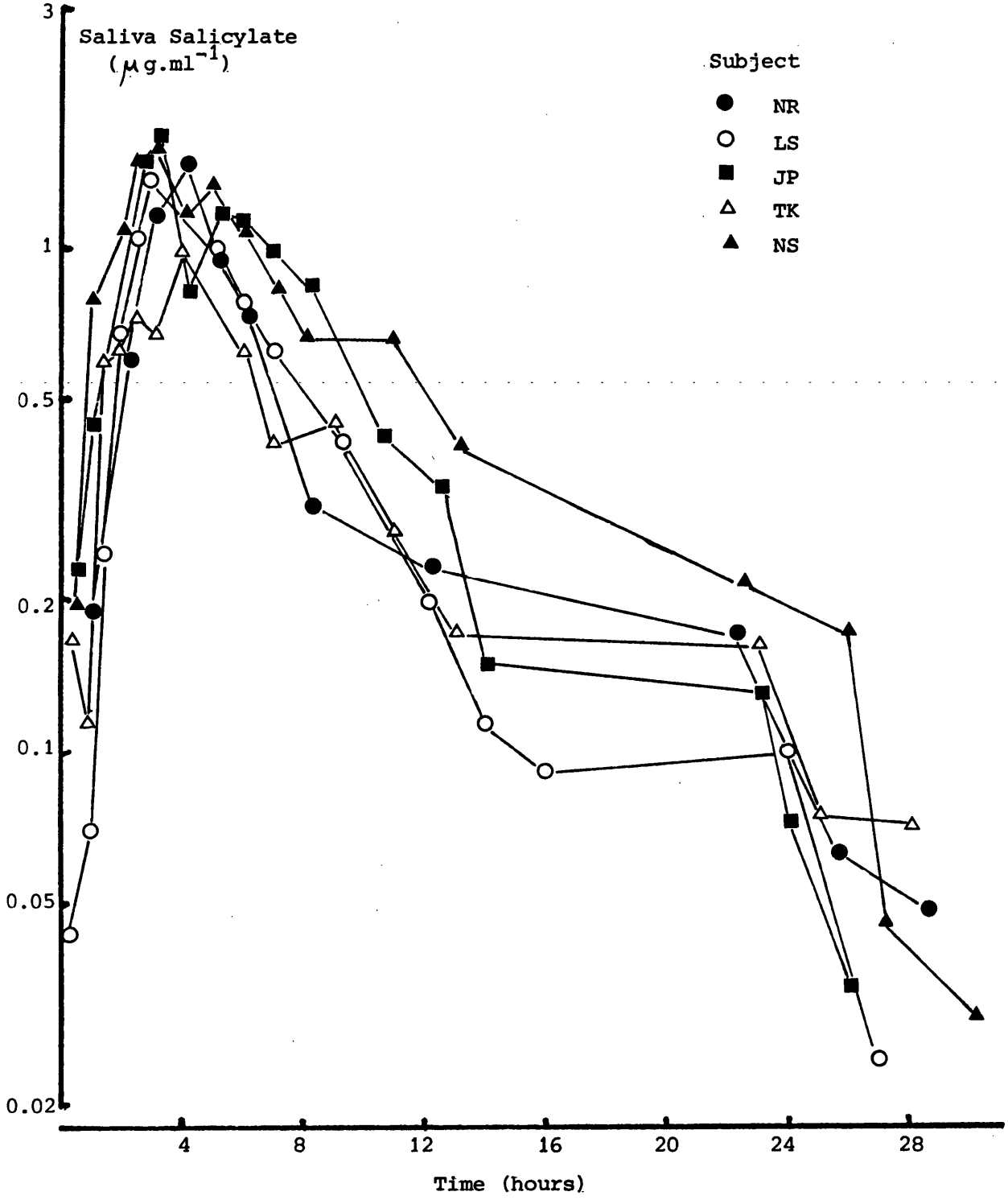


Fig. 3.2.16 Saliva Salicylate (log scale) vs Time Profiles For SSA Following Oral Administration In Hard Gelatin Capsules. Dose 600 mg.

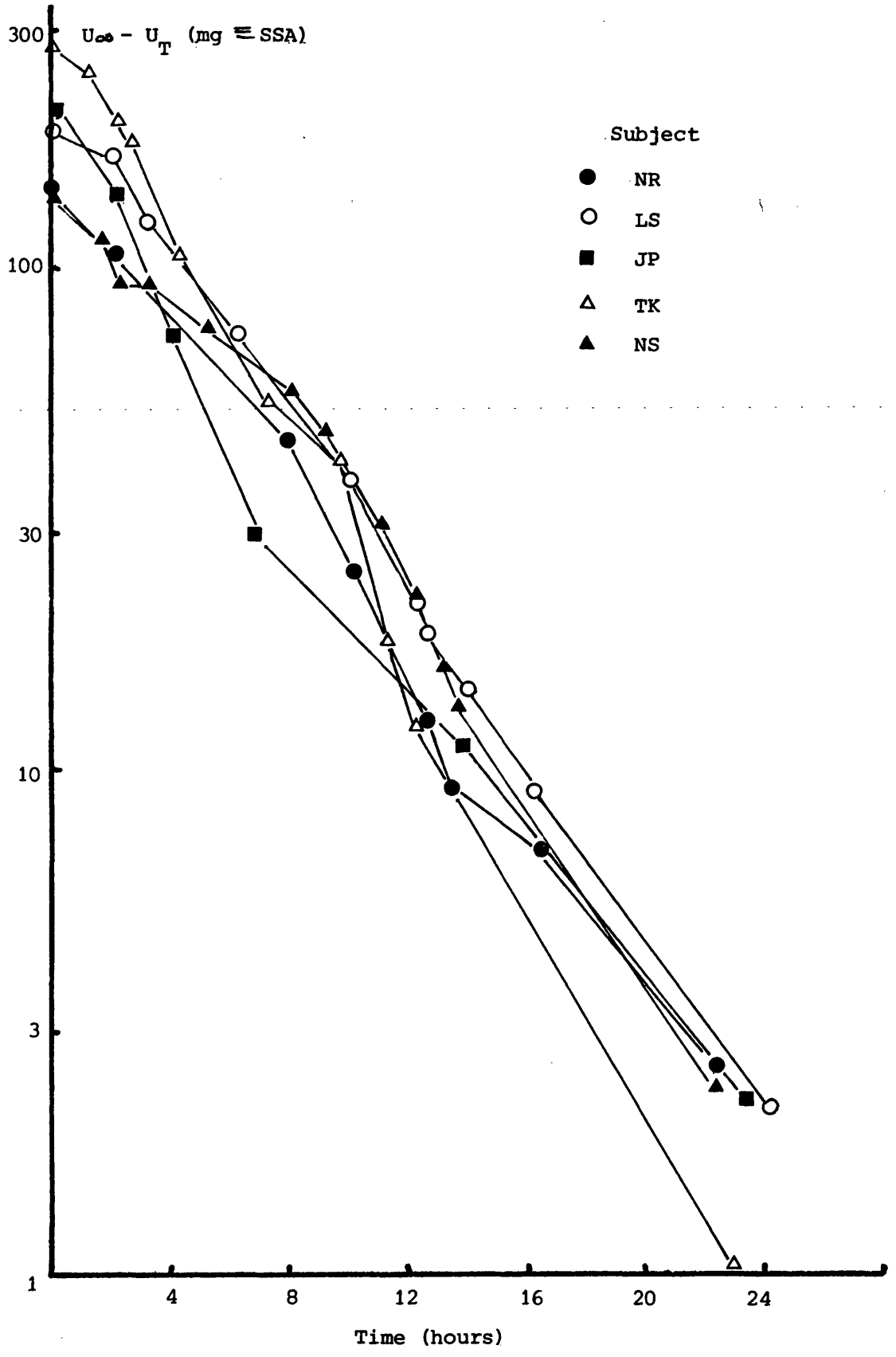


Fig. 3.2.17 Sigma-minus Plots Obtained From Five Subjects Following Oral Administration Of SSA (micronised) In Hard Gelatin Capsules. Dose 600 mg.

		Subject				
		NR	NS	LS	JP	TK
URINE	$k_{el} \pm SE\ (hr^{-1})$	0.1916 ±0.010	0.1831 ±0.011	0.1940 ±0.004	0.1951 ±0.013	0.2523 ±0.005
	r	0.992	0.984	0.998	0.991	0.998
	Half-life (mins)	217.0	227.1	214.3	213.1	164.8
	% Dose Excreted	23.7	23.3	30.8	34.7	47.3
SALIVA	$k_{el} \pm SE\ (hr^{-1})$	0.2308 ±0.050	0.1281 ±0.012	0.2151 ±0.007	0.2040 ±0.017	0.1752 ±0.023
	r	0.937	0.979	0.996	0.980	0.968
	Half-life (mins)	180.2	324.6	193.3	203.8	237.6

Table 3.2.11 Apparent First Order Elimination Rate Constants (k_{el}), Associated Half Lives and % Dose Excreted Determined From Urinary Excretion and Saliva Concentration Data Following Oral Administration of SSA in Capsules.

tion over the sleeping hours (16-24 hours post-administration). Closer examination of the corresponding sections of the 'Sigma-minus' plots reveals a decrease in the apparent first order elimination rate constant 12-16 hours after dosing. The suggestion therefore, is that the discrepancy between the k_{e1} values for the two studies could be due to a much prolonged absorption or a secondary (somnambulant) absorptive phase of salicylate from the larger dose.

A further explanation for the observed differences in the elimination rate constants is the concentration range over which they were calculated. In the former study (60 mg SSA) elimination rate constants were derived from linear terminal portions of the 'Sigma-minus' plots over the 10-1 mg range for SSA unexcreted. In the later study (600 mg SSA) the corresponding range of the 'Sigma-minus' plots were from 100-10 mg SSA unexcreted. It has previously been suggested (Section 3.1), and shown for aspirin (Fig. 3.2.6 and accompanying discussion), that deviations from true first order elimination kinetics is a characteristic of salicylate disposition a function which is capacity-limited. In view of this it can be postulated that the slower elimination observed for the higher dose could be a manifestation of capacity-limited kinetics; a situation that could be exacerbated by the prolonged time-course of SSA absorption.

3.2.7 GENERAL CONCLUSIONS FROM HUMAN STUDIES

The similarity in the parameters of salicylate elimination derived from salivary and urinary excretion data, but not from plasma, indicates the possibility of differences between salicylate

analysis in saliva and urine with that for plasma. Since only non-protein-bound salicylate is able to be transferred from the blood into the salivary gland the respective assays must therefore also measure non-bound material. The discrepancy between saliva and plasma elimination data and possibly the time-related change in the salicylate concentration ratio between the two body fluids could be attributed to the likelihood that the salicylate measured in plasma was an over-estimation resulting from displacement of protein-bound material during the acidification and ether extraction procedures. However, the elucidation of analytical problems and subsequent absorption studies in man were not pursued for a number of reasons. Complete characterisation of the plasma and saliva salicylate profile designed to provide a comprehensive saliva/plasma correlation was not feasible owing to the extended length of time over which subjects would be required to give blood samples. Furthermore, most volunteers were available for only a limited number of studies; this was due to their reluctance to be subjected to the experimental conditions for a series of routine and extended studies. It was extremely unlikely that volunteers would collaborate in intravenous administration studies that would be essential for the elucidation of some basic pharmacokinetic parameters. It was therefore necessary to employ an alternative experimental animal model that could be routinely used for biopharmaceutical investigations. All future studies were therefore performed using the rat since various limitations precluded the use of any other species.

3.3 BIOAVAILABILITY STUDIES IN THE RAT

The limitations associated with the long-term use of a group of human volunteers necessitated that an alternative *in vivo* model was found that would allow routine evaluation of physical, chemical and formulatory influences on the gastrointestinal absorption of SSA. Barr (1972) has reviewed the use of several *in situ* perfused and closed intestinal preparations that have been successfully employed to study drug absorption from the gut in the anaesthetised animal and has concluded that, the greatest potential for differentiating between formulatory changes *in vivo* was offered by measuring the appearance of drug in the blood, as opposed to following its loss from the intestinal lumen. While the *in situ* approach would have been useful for studies on drug absorption from solution, investigations where the drug is introduced in a suspended or solid dosage form can be complicated by sedimentation throughout the perfusing system or in 'stagnant' sections of the perfused intestinal segment. Further complications arising from such anaesthetised preparations can be anticipated from changes in mesenteric blood flow due to the depth and type of anaesthesia. In view of these considerations bioavailability studies on suspended SSA systems were performed in the conscious animal by monitoring plasma or urine salicylate concentrations following oral administration.

3.3.1 ANIMALS

White albino male rats of the Wistar CFY strain (Anglia Laboratory Animals, Huntingdon) were housed in groups of six and allowed free access to water and food (Oxoid diet 41B) between experiments.

3.3.2 GENERAL EXPERIMENTAL PROTOCOL

To allow the collection of sufficient samples of blood over a time period adequate to describe salicylate absorption and elimination (16-24 hours) it was necessary to use rats of the 450-550 g weight range. Each absorption study was performed on a group of six animals and, where possible (e.g. for coprecipitate/admixture comparisons), a cross-over design was implemented. However, due to the large number of studies envisaged for any single objective it was not possible to employ a complete cross-over since, throughout an extended period animal weight gain would be excessive. For each study, animals were fasted over night (approx. 16 hours) prior to dosing and the fast continued for a further four hours; water was allowed ad libitum.

3.3.3 DOSAGE

For all experiments dosing was on a mg (equivalent SSA) kg^{-1} body weight basis. The SSA:PVP, CA and DOCA coprecipitates and admixtures were dosed according to their SSA content (See Sections 2.13.1.1 and 2.13.1.2).

3.3.3.1 ORAL ADMINISTRATION was achieved by direct oesophageal intubation of the manually-restrained animal, using an adapted, blunt, stainless steel, serum needle fitted to the appropriate size syringe.

3.3.3.2 INTRAVENOUS ADMINISTRATION of drug solutions was carried out, under light ether anaesthesia, by bolus injection via the dorsal vein of the penis. Anatomical examination of the cardiovascular system of the rat (Greene, 1935) and the studies of Nightingale

and Mouravieff (1973) show that injection by this route avoids the enterohepatic circulation and that administered drug enters the systemic cardiovascular system via the inferior vena cava. This method was found particularly suited to large rats since injection via the more commonly used tail route is difficult to perform in animals over 300 g. Intravenous injection via the penis was quick, efficient, reliable and produced negligible, overt discomfort to the animals upon recovery from anaesthesia.

3.3.4. COLLECTION OF RAT URINE

Following dosing, animals were singly housed in a round (10" diameter) cage, fitted with a ½" mesh floor, placed over a 12" diameter polyethylene funnel equipped with a glass urino-faecal separator. Urine and faeces were therefore collected separately, the latter being discarded. Urine was allowed to drain into a 50 ml measuring cylinder and at 4, 24 and 48 hours after dosing each funnel was thoroughly rinsed, the washings being retained and added to the collected urine. An aliquot of the measured volume of urine (+ washings) was stored at -15^{o} prior to assay for total salicylate.

3.3.5. COLLECTION OF RAT PLASMA

Plasma used in the preparation of salicylic acid standards was obtained by cardiac puncture from animals, under light ether anaesthesia. Blood, withdrawn into a heparinised syringe, was centrifuged at 3000 $rev.min^{-1}$ in glass centrifuge tubes for 15 minutes and the plasma removed by pasteur pipette prior to storage at -15^{o}.

Throughout the absorption studies 0.6-0.7 ml samples of whole blood were taken from the tip of the tail of each animal at specified times. For this collection animals were placed in all-metal restraining cages that allowed minimal movement of the animal and free access to the tail. The tip of the tail (approximately 2 mm) was removed with a scalpel blade and blood collected into a 0.75 ml polyethylene micro-centrifuge tube (Sarstedt, Leicester) containing approximately 10 μl heparin (5000 units ml^{-1}). Blood flow through the tail was facilitated by maintaining the animals at a room temperature of 25-29^{o} and, drainage of blood into the collecting vessel was aided by gentle stroking of the tail with the fingers in a base-to-tip direction. Plasma was obtained by centrifugation, 0.2 ml aliquots of which were removed by automatic pipette (Oxford Laboratories) and stored at -15^{o} prior to assay.

This method of obtaining plasma samples imposed little discomfort on the animals and allowed repeated sampling over a 24 hour period. To ensure complete salicylate excretion and adequate healing of the tail, etc, a one week period was allowed between any consecutive studies on the same animal.

Familiarity with the time required to obtain a plasma sample from each of six animals enabled dosing to be staggered such that the times of sampling, following either oral or intravenous administration, were similar ($\pm$ 3 minutes) for each of the animals within a single group.

3.3.6 ASSAY OF SALICYLATE IN RAT URINE

The method employed was the same as that described previously (See 3.2.2.1). Values of slope and intercept for duplicate salicylic acid standard (4-200μg.ml^{-1}) calibration plots (Fig. 3.3.1) are given in Table 3.3.1 with their respective standard errors and correlation coefficients. Comparison of the duplicates by the Student 't' test shows them not to be significantly different; therefore, the data was pooled and submitted to linear least-squares regression analysis to provide the equation used in all future determinations of the salicylate content of the experimental samples.

	Slope $\pm$ SE ($\times 10^{-3}$)	Intercept $\pm$ SE ($\times 10^{-3}$)	r	't' Test
A	5.248 $\pm$ 0.035	3.964 $\pm$ 0.046	0.999	t_{obs} = 0.294
				n = 14
B	5.235 $\pm$ 0.027	2.141 $\pm$ 2.339	0.999	p' = 0.05
				t_{tab} = 2.281
Pooled	5.241 $\pm$ 0.021	3.052 $\pm$ 1.830	0.999	

Table 3.3.1 Comparison of Duplicate Salicylic Acid Calibration Curves in Rat Urine.

From Table 3.3.1 the slope of the Beer-Lambert plot gave an $E^{1\%}_{1cm}$ of 52.41 and a molar absorbance coefficient of 723.8 for salicylic acid. As for the human studies the total salicylate detected in rat urine was calculated from a knowledge of the concentration per

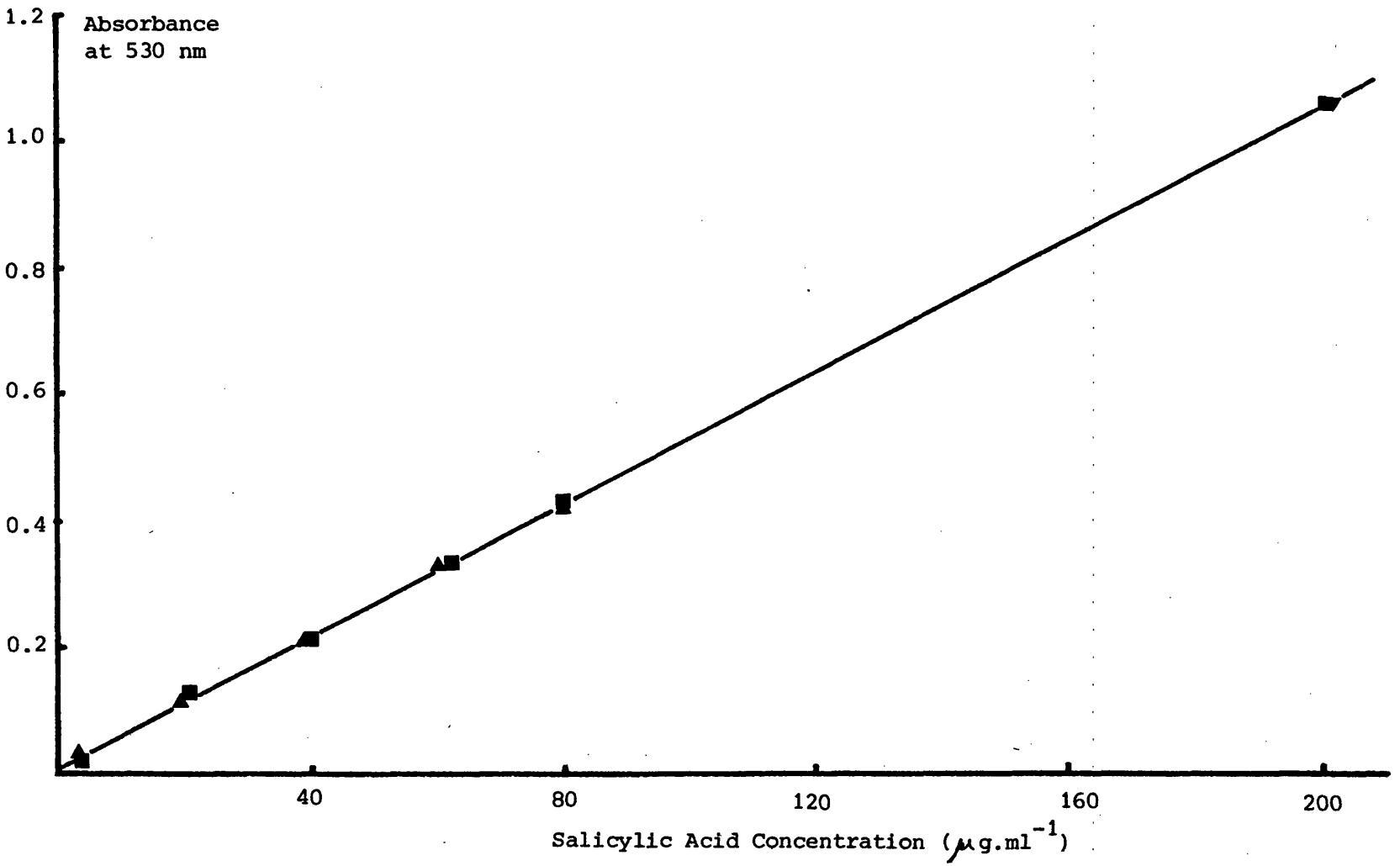


Fig. 3.3.1 Beer-Lambert Plot For Salicylic Acid Duplicates In Rat Urine.

ml, the dilution (if any) and the volume voided. Total salicylate excreted was expressed as SSA equivalents to calculate the percentage of the dose excreted for each collection period.

To demonstrate the efficiency of the degradation of SSA and salicylate metabolites by autoclaving under alkaline conditions, SSA was intravenously administered at a 20 $mg.kg^{-1}$ dose to a group of six rats, the urine collected as a single batch for 48 hours and assayed as described. The mean % dose excreted was calculated to be 95.25 ± 1.41. This percentage of the dose excreted following intravenous administration provides a bioavailability standard for future urinary excretion studies where the same dose of SSA is administered.

3.3.7 ASSAY OF SALICYLATE IN RAT PLASMA

The total salicylate content of plasma samples was assayed according to the flow diagram in Fig. 3.3.2. Standard salicylic acid samples (1-125 $\mu g.ml^{-1}$) were prepared in plasma in triplicate and assayed by the above procedure. Plots of relative fluorescence vs concentration are shown in Fig. 3.3.3 and the three calibration curves were compared by a Student 't' test using the data given in Table 3.3.2. These triplicate curves were shown not to be significantly different (Table 3.3.3), therefore the data was pooled and submitted to linear least-squares regression to provide the equation used to calculate the salicylate content of experimental samples.

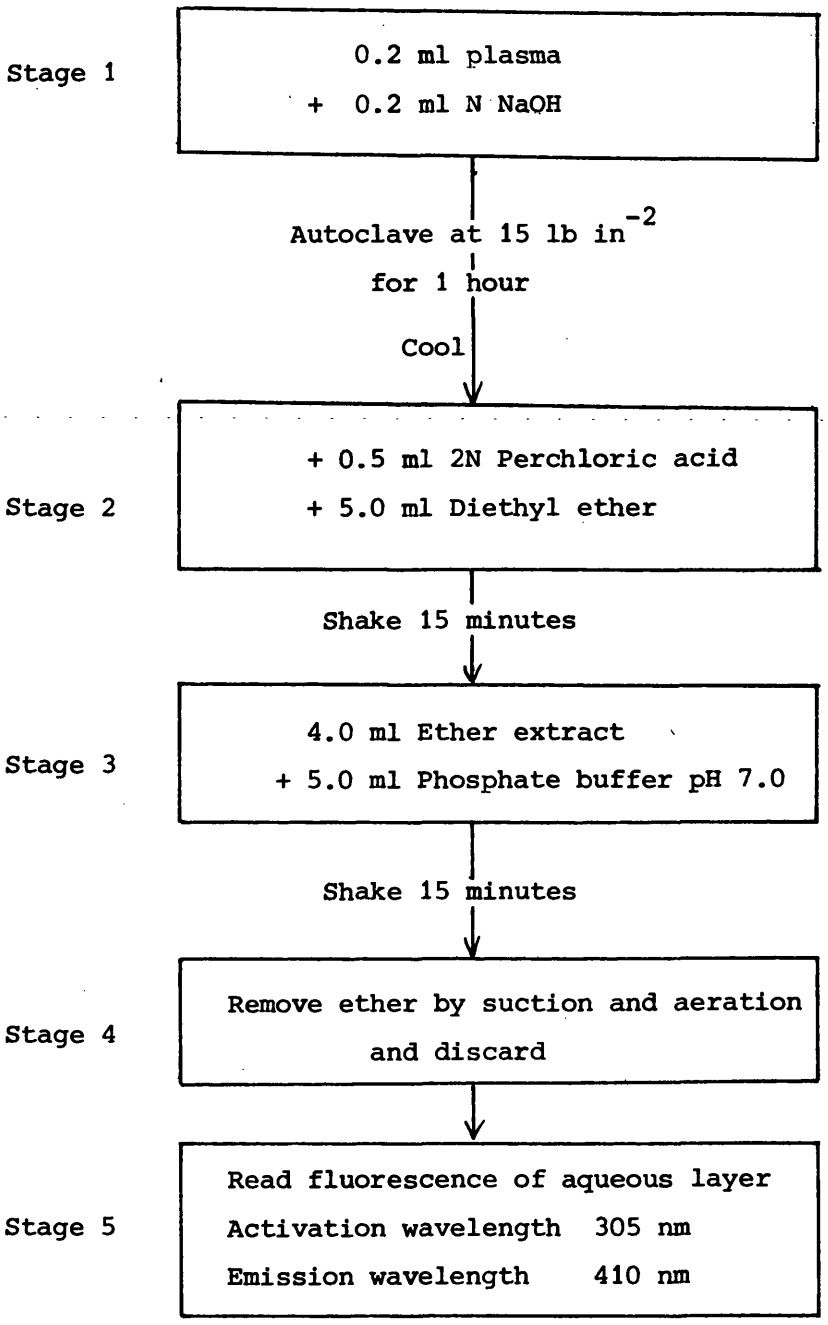


Fig. 3.3.2 Flow Diagram For the Assay of Salicylate in Rat Plasma

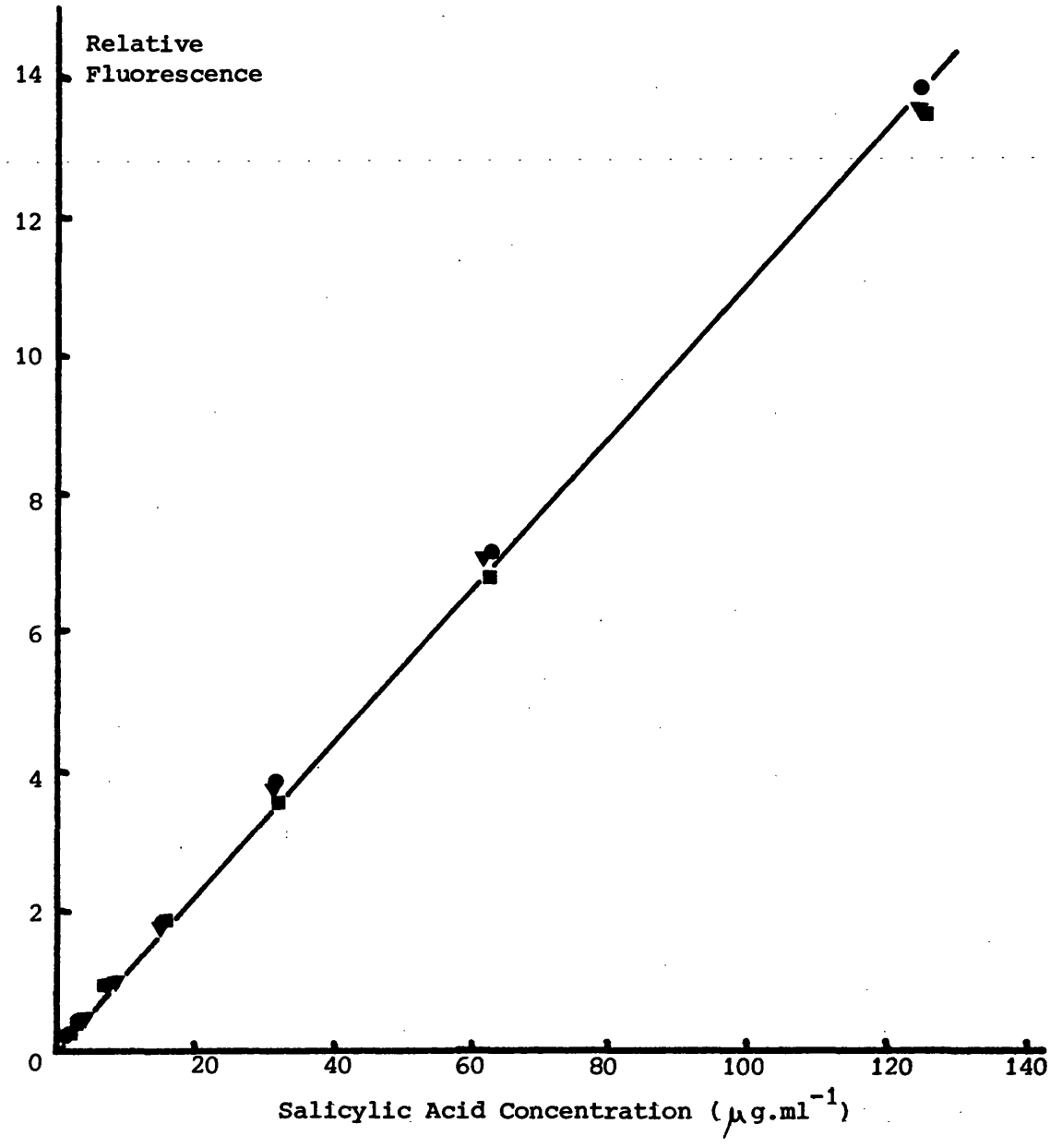


Fig. 3.3.3 Triplicate Standard Fluorescence Calibration Plots For Salicylic Acid In Rat Plasma.

	Slope ± SE ($x10^{-1}$)	Intercept ± SE ($x10^{-1}$)	r
A	1.120 ± 0.012	0.978 ± 0.610	0.999
B	1.106 ± 0.126	4.935 ± 6.389	0.963
C	1.082 ± 0.050	0.956 ± 0.275	0.999
Pooled	1.103 ± 0.040	2.290 ± 2.019	0.986

Table 3.3.2 Data for Triplicate and Pooled Salicylic Acid Standard Curves in Rat Plasma

Comparison	t_{calc}	t_{tab}	N	p'
A vs B	0.111	2.179	16	0.05
A vs C	0.739	2.179	16	0.05
B vs C	0.177	2.179	16	0.05

Table 3.3.3 Student 't' Test Comparison Between Triplicate Salicylic Acid Standard Curves in Rat Plasma

3.3.8 REPRESENTATION OF DATA

3.3.8.1 URINARY STUDIES

All data is given as a percentage of the dose (equivalent SSA) excreted 4, 24 or 48 hours after drug administration.

3.3.8.2 PLASMA STUDIES

Data is presented graphically as plots of mean ($\pm$ SE) salicylate concentration on a linear or a logarithmic scale vs time. From these plots it was possible to identify sections where either first or zero order kinetics could be applied and thereby isolate the appropriate data points for each animal that could be submitted to regression analysis to obtain the respective apparent first or zero order rate constants. Zero order rate constants obtained from intravenous experiments were used to construct a Lineweaver-Burk type of plot according to equation (44) from which values of V_{max} and K_m, for concomitantly-occurring capacity-limited metabolic processes, were interpolated. Apparent first order elimination rate constants and corresponding half-lives were calculated and used throughout for comparing the influence of formulation, etc on salicylate elimination.

Further comparison between studies was achieved by the use of the area under the curve (AUC) of the plasma salicylate concentration vs time profile, calculated according to the trapezoidal rule by use of the program given as Appendix III. Estimates of bio-availability were calculated by comparison of AUC data for each of the experimental formulations with that for the corresponding AUC derived from intravenous or oral data according to equation (46).

Examination of the AUC and k_{e1} data for variance attributable to formulatory changes, as opposed to those of animal origin, was achieved by a variance ratio test and, where any significant effect was evident a Student 't' test between each of the treatments was carried out.

3.3.9 INTRAVENOUS ADMINISTRATION OF SSA

The first studies performed in the rat were investigations of the influence of dose on the plasma level vs time profiles following intravenous administration. The aims were to gain information regarding the disposition of total salicylate, to identify and possibly characterise any capacity-limited metabolic processes and to provide a bioavailability standard that could be employed as a reference for equivalent oral doses of SSA.

It should be made clear at this stage that all plasma and urine studies in the rat were based on the measurement of total salicylate which, by definition, includes SSA, salicylic acid and their respective metabolites (See Fig. 3.6). Although inferences regarding the specific pharmacokinetic behaviour of SSA, salicylic acid or any of the metabolites alone should not be made from such data, it does however, provide the means for assessing the efficiency of absorption and subsequent comparison between routes of administration, and between dosage forms of various formulations, particle size range, etc.

The poor aqueous solubility of SSA presented difficulties in the preparation of a suitable vehicle for its intravenous (iv) injection. Aqueous solutions incorporating propylene glycol as

a cosolvent were immediately discounted due to the haemoglobinurea observed in a series of preliminary experiments. This type of haemolytic response could have been expected to have severe adverse effects, not only on the well-being of the animal, but also on drug disposition and assay. A suitable aqueous vehicle was obtained by the complete dissolution of SSA in 5 ml of 95% ethanol and subsequent dilution to 25 ml with Sørensen's phosphate buffer, pH 7.4. The pH of the final solution was determined to be 7.4 and thus unchanged upon dilution. This vehicle allowed i.v. bolus injection of SSA in doses of 50 $mg.kg^{-1}$ and below. For each dose level only the initial weight of SSA was adjusted so that animals received identical amounts of ethanol and buffer salts (on a $mg.kg^{-1}$ basis). Other experimental conditions were as previously described in Section 3.3.2.

3.3.9.1 INFLUENCE OF DOSE

I.v. doses of 50, 30, 20, 10 and 2.5 $mg.kg^{-1}$ were given by single bolus injection via the dorsal vein of the penis. Blood samples, taken at zero time and at appropriate intervals after dosing were assayed for total salicylate. The linearity of the mid-profile sections of plots of mean ($\pm$ SE) plasma salicylate concentration vs time (Fig. 3.3.4) for the five dosage levels is indicative that zero order processes predominate. This is particularly evident for the higher doses.

Wagner (1975) has suggested that if the pharmacokinetic processes obey first order kinetics then the ratios between the plasma concentration (C) and the initial concentration (C_o) should be the same at a given time for different doses; ie plots of

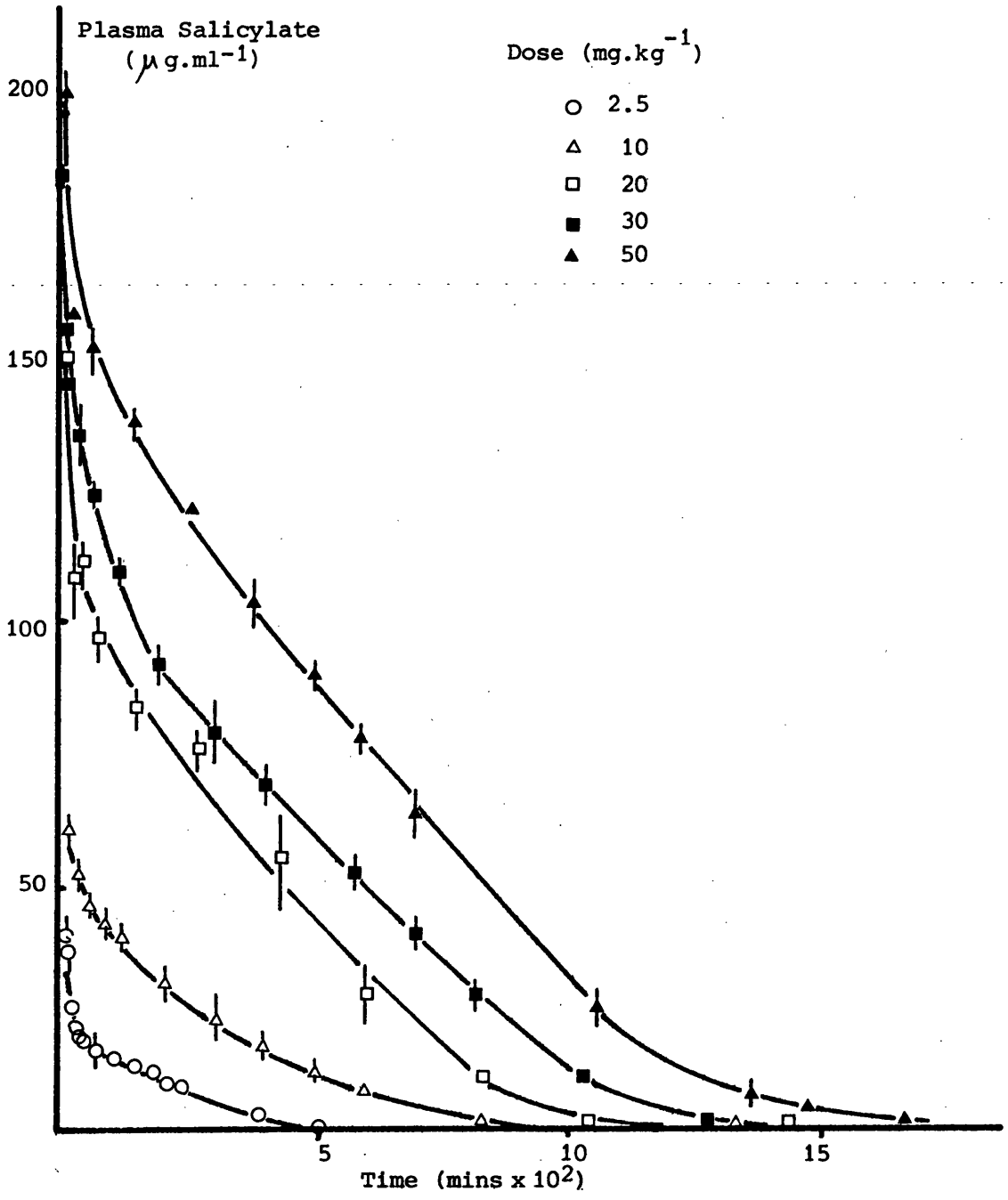


Fig. 3.3.4 Plasma Salicylate Profiles For SSA Following Intravenous Administration - Influence Of Dose.

C/C_o *vs* t should be superimposable. For the intravenous data presented in Fig. 3.3.4, the C_o values were calculated as the t = o intercept of a line defined by the first two data points. The lack of superimposition of the plots of C/C_o *vs* t (Fig. 3.3.5) is indicative of non-linearity in the pharmacokinetic processes occurring during salicylate disposition following i.v. injection of SSA at these five dosage levels. It is appropriate therefore, to propose that the overall disposition of SSA, as measured by total salicylate concentration in the plasma, exhibits capacity-limited kinetics that are probably metabolic in origin.

Consequently apparent zero order rate constants were calculated by subjective examination of the individual profiles at each dose level and submission of appropriate data points to linear least-squares regression analysis. The apparent zero order rate constants (k_o) for each dose are given in Table 3.3.4 with their respective reciprocals. Since it is valid to pool concomitantly-occurring first order rate constants to produce an "overall rate constant" and it has further been suggested that parallel zero order kinetic processes can similarly be pooled (Sedman and Wagner, 1972), it therefore seemed justifiable to construct a Lineweaver-Burk type plot ($^1/k_o$ *vs* ^{1}/Dose) from the data given in Table 3.3.4. From such a plot it would be possible to provide information regarding the "overall zero order rate constant" of salicylate metabolism following i.v. administration of SSA. The plot is shown in Fig. 3.3.6.

The reported method of constructing such double reciprocal plots (Wagner, 1973a) and other mathematical treatments of data that

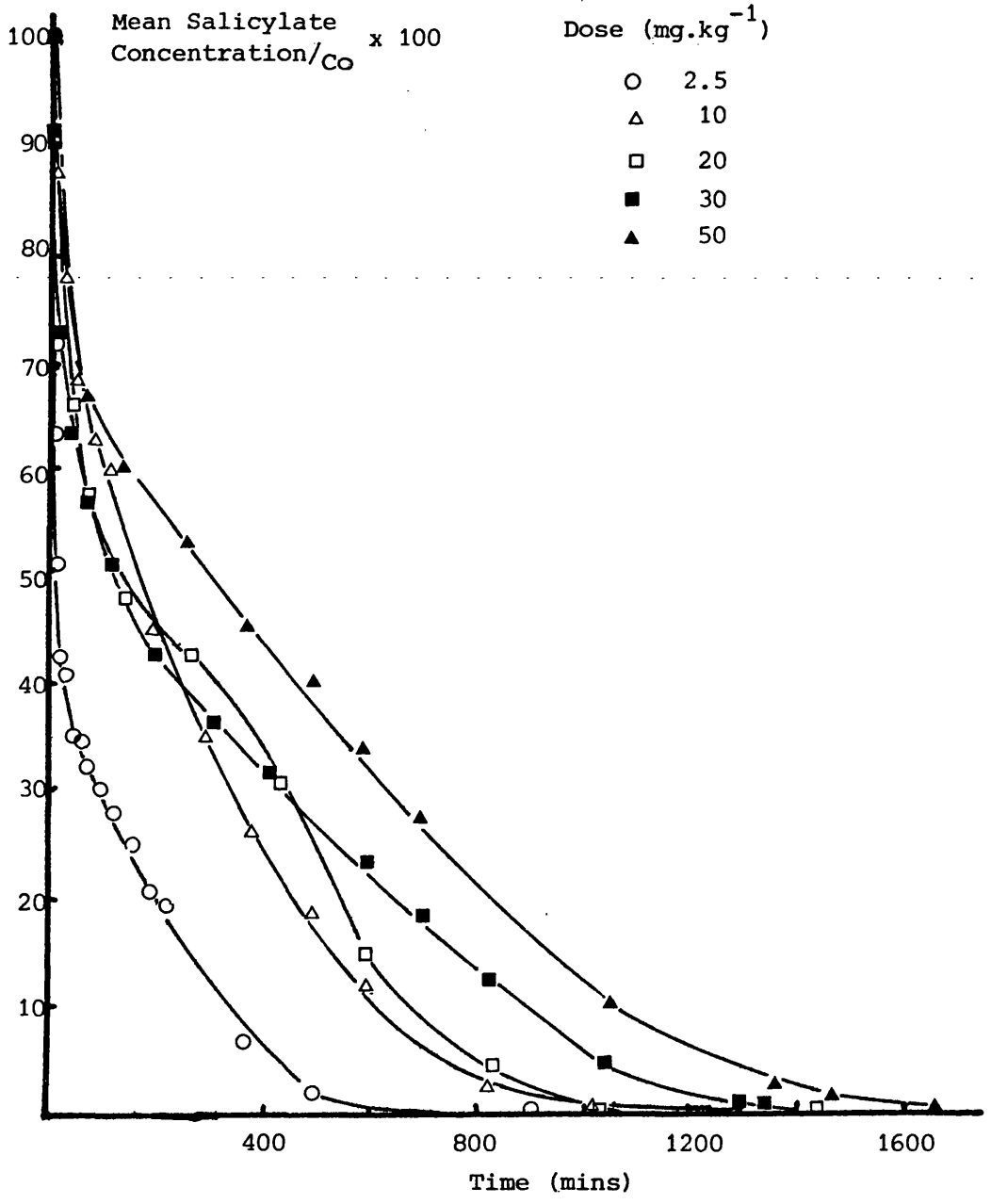


Fig. 3.3.5 Relationship Between Percentage Of Initial Concentration (t = 0) And Time Following Intravenous Administration Of SSA At Five Dosage Levels.

Dose ($mg.kg^{-1}$) / $^1/dose$ ($x10^{-2}$) ($kg.mg^{-1}$)	Apparent Zero Order Rate Constant (k_o) ± SE ($x\ 10^{-2}\ min^{-1}$)						
	$^1/k_o$						
	1	2	3	4	5	6	mean±SE
2.5	4.31±0.71	3.60±0.39	3.95±0.28	3.70±0.29	3.36±0.63	5.68±1.03	4.10±0.34
40.0	23.19	27.82	25.33	27.06	29.79	17.61	25.13±1.76
10.0	9.35±0.50	15.34±1.58	7.89±0.33	10.39±0.65	9.37±1.35	10.31±0.21	10.44±1.05
10.0	10.69	6.52	12.68	9.62	10.68	9.70	9.98±0.83
20.0	10.25±0.14	12.78±0.24	14.11±1.11	14.05±0.98	12.90±1.08	-	12.82±0.70
5.0	9.76	7.83	7.09	7.12	7.75	-	7.91±0.49
30.0	9.06±0.69	9.41±0.26	10.11±0.38	11.39±0.49	9.52±0.37	11.91±0.36	10.23±0.47
3.33	11.04	10.62	9.89	8.78	10.50	8.40	9.87±0.44
50.0	13.00±0.46	11.18±0.26	17.14±0.60	12.61±0.48	13.99±0.84	-	13.58±1.00
2.0	7.70	8.94	5.83	7.93	7.15	-	7.51±0.51

Table 3.3.4 Apparent Zero Order Elimination Rate Constants Following Intravenous Administration of SSA at Five Doses

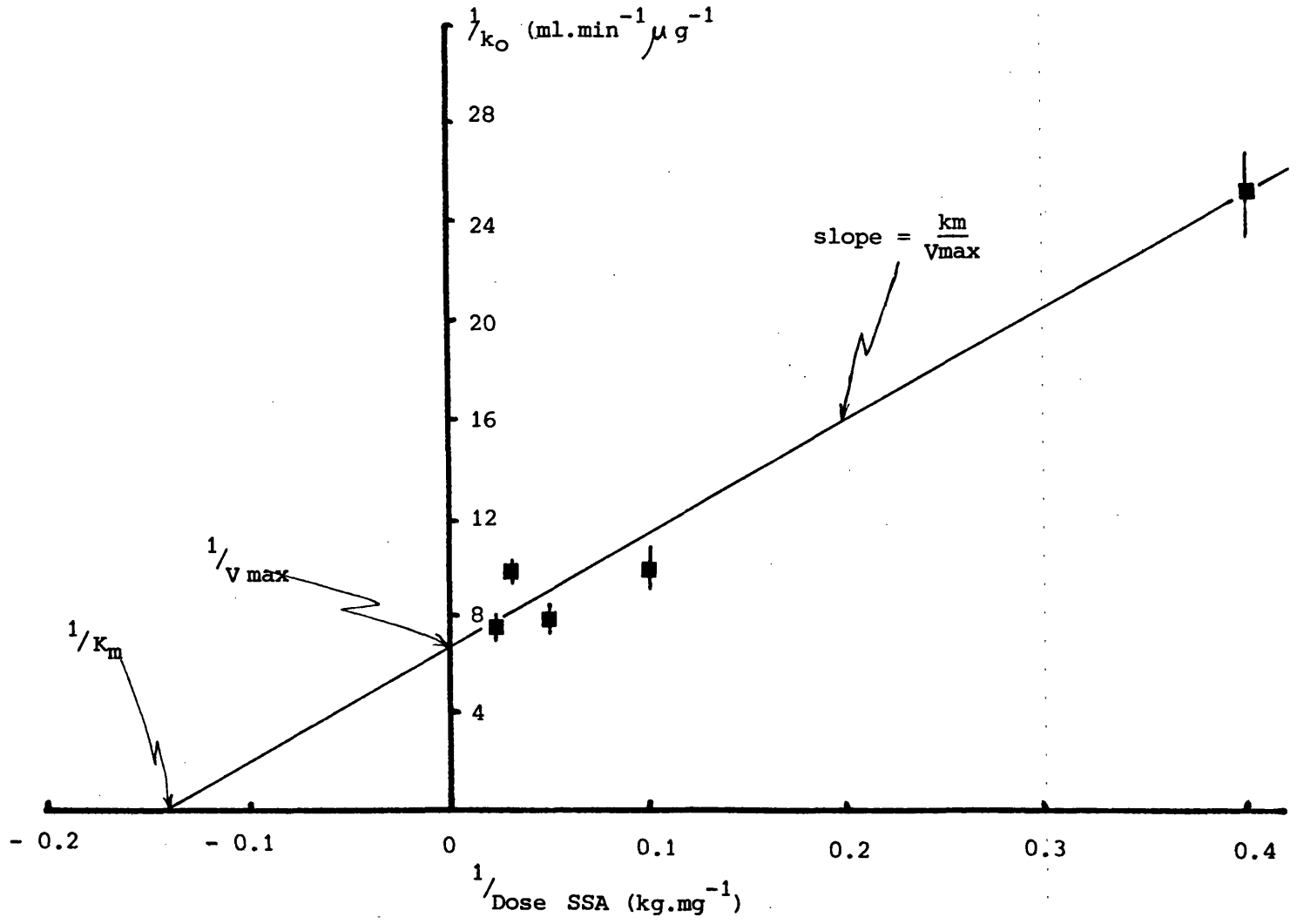


Fig. 3.3.6 Lineweaver-Burk Plot of $^1/_{ko}$ (the apparent zero order elimination rate constant) vs $^1/$ dose For SSA Following Intravenous Administration.

obey Michaelis-Menten kinetics (Martis and Levy, 1973; van Ginneken et al, 1974; Wagner, 1975) all depend on the dispositon of the drug complying with the one-compartment model. In view of the fact that the observed data for SSA, even at low doses, cannot be described by this model, the Lineweaver-Burk plot was constructed using reciprocal dose rather than reciprocal C_o values. From Fig. 3.3.6 values for K_m and V_{max} of 6.94 $mg.kg^{-1}$ and 0.1515 $\mu g.ml^{-1}min^{-1}$ respectively were calculated from the slope and intercepts of the line obtained by least-squares regression analysis. These values suggest that above i.v. doses of SSA greater than 7 $mg.kg^{-1}$ the metabolism of the salicylate in the rat will occur at an apparently constant rate. This value is in close agreement with that of 7 $mg.kg^{-1}$ quoted for salicylurate formation in the rat following intraperitoneal injection of sodium salicylate (Nelson et al, 1966) and suggests that the major capacity-limited route for SSA elimination maybe that of salicylurate formation from free salicylic acid.

When the dose $\gg$ K_m the rate of metabolite formation will be maximal, whereas when $K_m \gg$ dose this rate will be minimal and the Michaelis-Menten equation (44) reduces to:-

$$v_o = kC \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (48)$$

which shows v_o, the initial rate of salicylate elimination, to have a first order dependence on C, the dose, where k is the apparent first order elimination rate constant. When the plasma salicylate concentration has declined to such an extent that the influence of the capacity-limited processes are negligible, then

the overall elimination of salicylate should be described by first order kinetics. An examination of the experimental data for sections of the profile where first order kinetics can be applied was achieved by plotting the mean ($\pm$ SE) plasma salicylate concentration (log scale) vs time (Fig. 3.3.7). It is evident that the terminal elimination phase for each set of mean data exhibits first order linearity and apparent first order elimination rate constants (k_{el}) were calculated for each animal within each dose range. These are given in Table 3.3.5 with the corresponding half-lives and correlation coefficients. Examination of these rate constants by a variance ratio test (Table 3.3.5a) shows that the size of the intravenous dose has no significant influence on the overall first order salicylate elimination rate constant.

Source of Variation	Sum Sq.	Degrees of Freedom	Mean Sq.	F Value	F Ratio	F Tabulated
Dose	31.26	4	7.814	2.097		F^{4}_{5} = 5.19
Animal	26.23	5	5.246	1.408	1.489	p' = 0.05
Residual	63.36	17	3.727			
Total	120.8	26				

Table 3.3.5a Variance Ratio Test for SSA Following Intravenous Administration At Five Dosage Levels - Elimination.

Area under the plasma salicylate concentration vs time profile (AUC) was calculated for each animal at each dose level (Table 3.3.6) and Fig. 3.3.8 shows that AUC and dose are linearly related

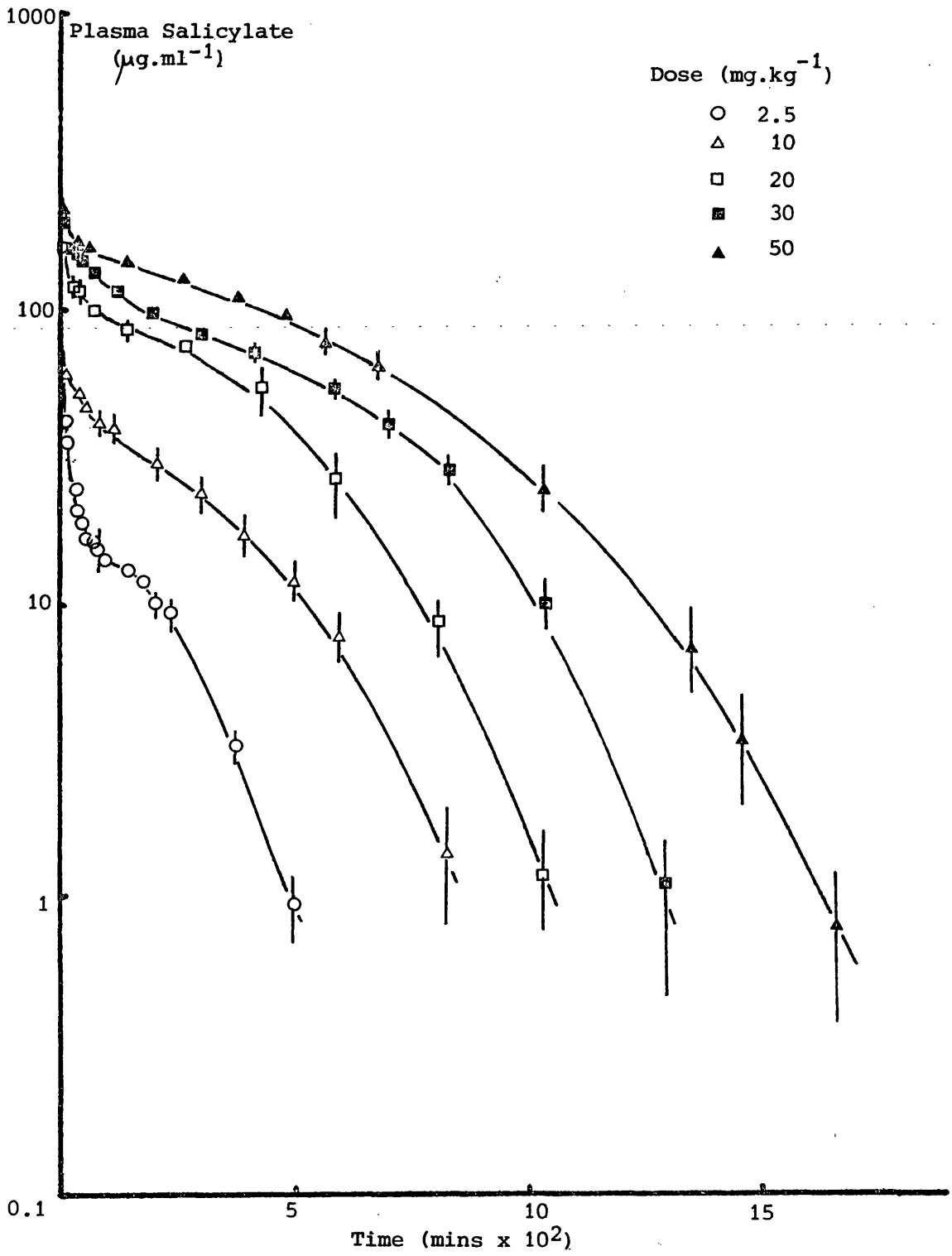


Fig. 3.3.7 Plasma Salicylate (log scale) vs Time Profiles For SSA Following Intravenous Administration - Influence Of Dose.

Dose ($mg.kg^{-1}$)		1	2	3	4	5	6	mean ± SE
2.5	k_{el} ± SE	6.410	11.779	4.624	8.806	8.085	10.973	8.446
	($x10^{-3}min^{-1}$)	±0.47	±1.19	±0.29	±0.52	±1.15	±1.68	±1.103
	$t_{½}$ mins	108	59	150	78	86	63	80
	r	0.990	0.995	0.990	0.997	0.990	0.977	-
10	k_{el} ± SE	8.861	3.428	4.105	-	4.519	5.939	5.370
	($x10^{-3}min^{-1}$)	±0.07	±0.18	±0.31	-	±0.60	±0.70	±0.965
	$t_{½}$ mins	78	202	169	-	153	117	129
	r	0.999	0.996	0.997	-	0.991	0.993	-
20	k_{el} ± SE	5.246	4.477	4.746	7.485	7.071	-	5.805
	($x10^{-3}min^{-1}$)	±0.37	±0.20	±0.31	±2.59	±1.44	-	±0.617
	$t_{½}$ mins	132	155	146	93	98	-	119
	r	0.991	0.998	0.991	0.945	0.980	-	-
30	k_{el} ± SE	4.881	7.622	4.449	7.606	8.309	4.548	6.236
	($x10^{-3}min^{-1}$)	±0.43	±1.75	±0.32	±0.72	±2.21	±0.41	±0.799
	$t_{½}$ mins	142	91	156	91	83	152	111
	r	0.996	0.975	0.995	0.996	0.966	0.996	-
50	k_{el} ± SE	8.551	7.048	4.283	5.960	6.026	-	6.374
	($x10^{-3}min^{-1}$)	±1.26	±1.63	±0.59	±0.18	±0.39	-	±0.702
	$t_{½}$ mins	81	98	162	116	115	-	108
	r	0.989	0.974	0.991	0.999	0.998	-	-

Table 3.3.5 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Intravenous Administration of SSA at Five Doses.

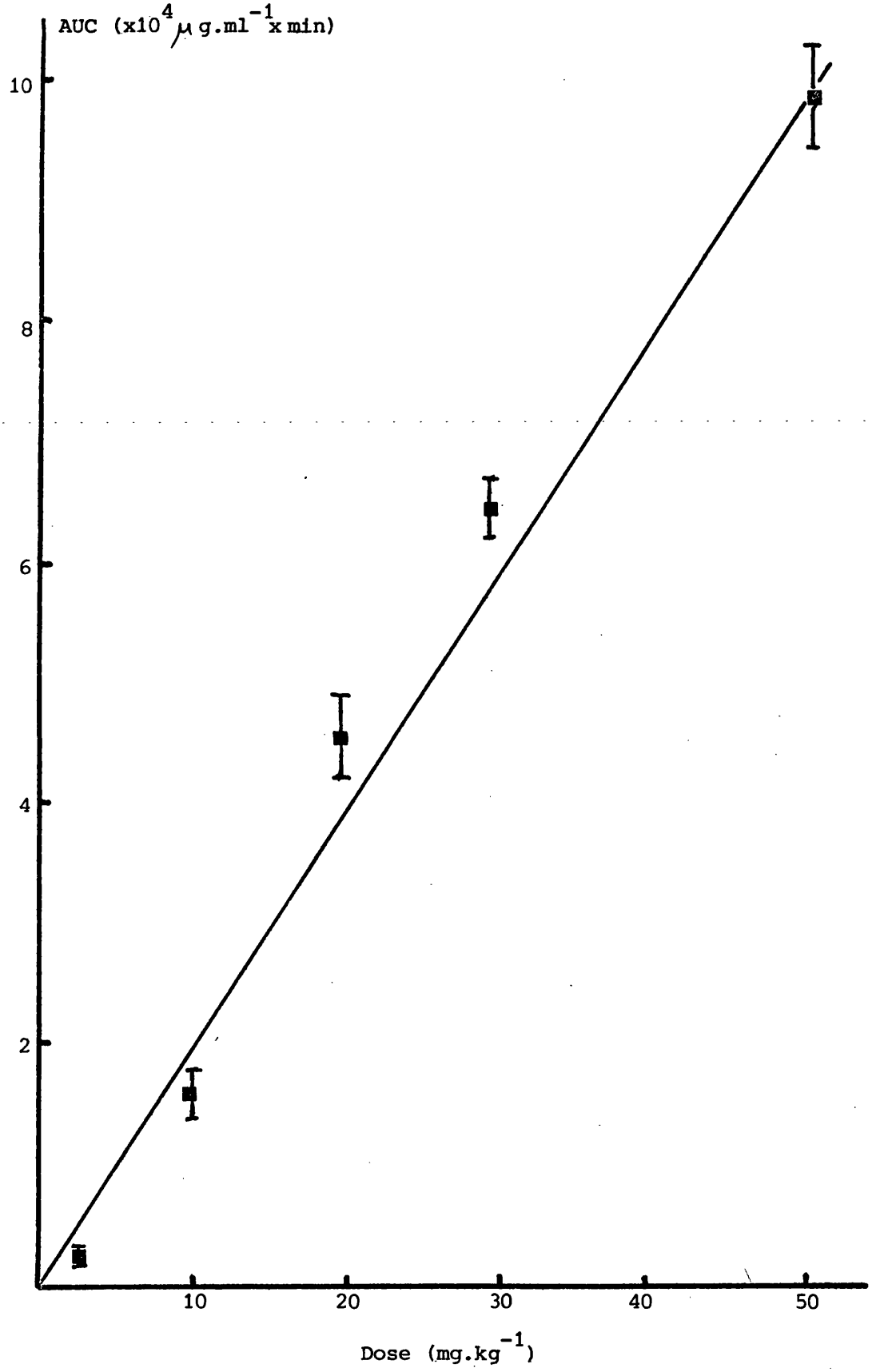


Fig. 3.3.8 Relationship Between Area Under The Plasma Level Curve (AUC) And Dose For SSA Following Intravenous Administration.

(r = 0.981). Such a linear relationship is not unexpected for measurements of total drug (unchanged drug and all metabolites, both free and protein-bound) even when capacity-limited processes are occurring. If AUC vs dose plots for unchanged drug, or any one of the metabolites, were made then the relationship would be altered and should show dose dependancy. Such a plot for AUC of unchanged drug would, however, be linear provided capacity-limited processes played an insignificant role in drug clearance. Conversely, the AUC vs dose relationship for any metabolite formed by a capacity-limited system would manifest an asymptomatic value, above which, increases in dose would not result in a corresponding increase in the AUC for that metabolite.

Intravenous Dose ($mg.kg^{-1}$)				SSA
2.5	10	20	30	50
4391.3	16160.7	37407.2	52300.0	108393.3
4828.7	12025.3	40857.2	71131.1	90837.1
4605.6	11772.6	38754.5	69884.0	90524.1
4085.0	17151.5	54439.4	64036.6	101662.3
5252.2	20976.6	55455.1	69202.4	103187.6
4254.6	18406.3	-	63137.0	-
4569	16082	45383	64948	98921
Mean ± SE ± 173	± 1478	± 3947	± 2857	± 3545

Table 3.3.6 Area Under the Plasma Salicylate-Time Profile ($\mu g.ml^{-1} \times min$) Following Intravenous Administration of SSA at Five Doses.

Although intravenous data provides the ideal standard for comparing drug bioavailability of other dosage forms or routes

of administration it has been stated that relative measurements of bioavailability can be achieved with reference to an oral dosage form, ie solution or proprietary product, etc. To examine the two approaches the gastrointestinal absorption of SSA from a solution was studied.

3.3.10 ABSORPTION OF SSA FROM SOLUTION

SSA was dissolved in Sørensen's phosphate buffer, pH 7.4, to give a concentration of 2 $mg.ml^{-1}$, this was administered by oral intubation at a dose of 1 $ml.100\ g^{-1}$ body weight, giving 20 $mg.kg^{-1}$.

The resultant mean plasma salicylate ($\pm$ SE) profiles are given in Figs. 3.3.9 and 3.3.10 for plots of salicylate concentration and salicylate concentration (log scale) vs time respectively. Elimination half-lives and apparent first order elimination rate constants (k_{el}) are given in Table 3.3.7 with AUC data.

	Elimination Data			AUC
	$k_{el} \pm$ SE ($x10^{-3}min^{-1}$)	$t_{½}$ (mins)	r	($\mu g.ml^{-1}$ x min)
	5.678 ± 0.24	122	0.996	24162.2
	6.504 ± 0.23	107	0.999	24718.0
	6.704 ± 0.29	103	0.998	26947.5
	6.630 ± 0.34	105	0.997	23249.0
	7.451 ± 0.69	93	0.996	23472.7
	7.040 ± 1.21	99	0.986	22995.3
Mean ± SE	6.668 ± 0.242	105 ± 4	-	24257.5 ± 596.8

Table 3.3.7 Apparent First Order Elimination Rate Constant (k_{el}), Half Life ($t_{½}$) and AUC Following Oral Administration of SSA in Solution.

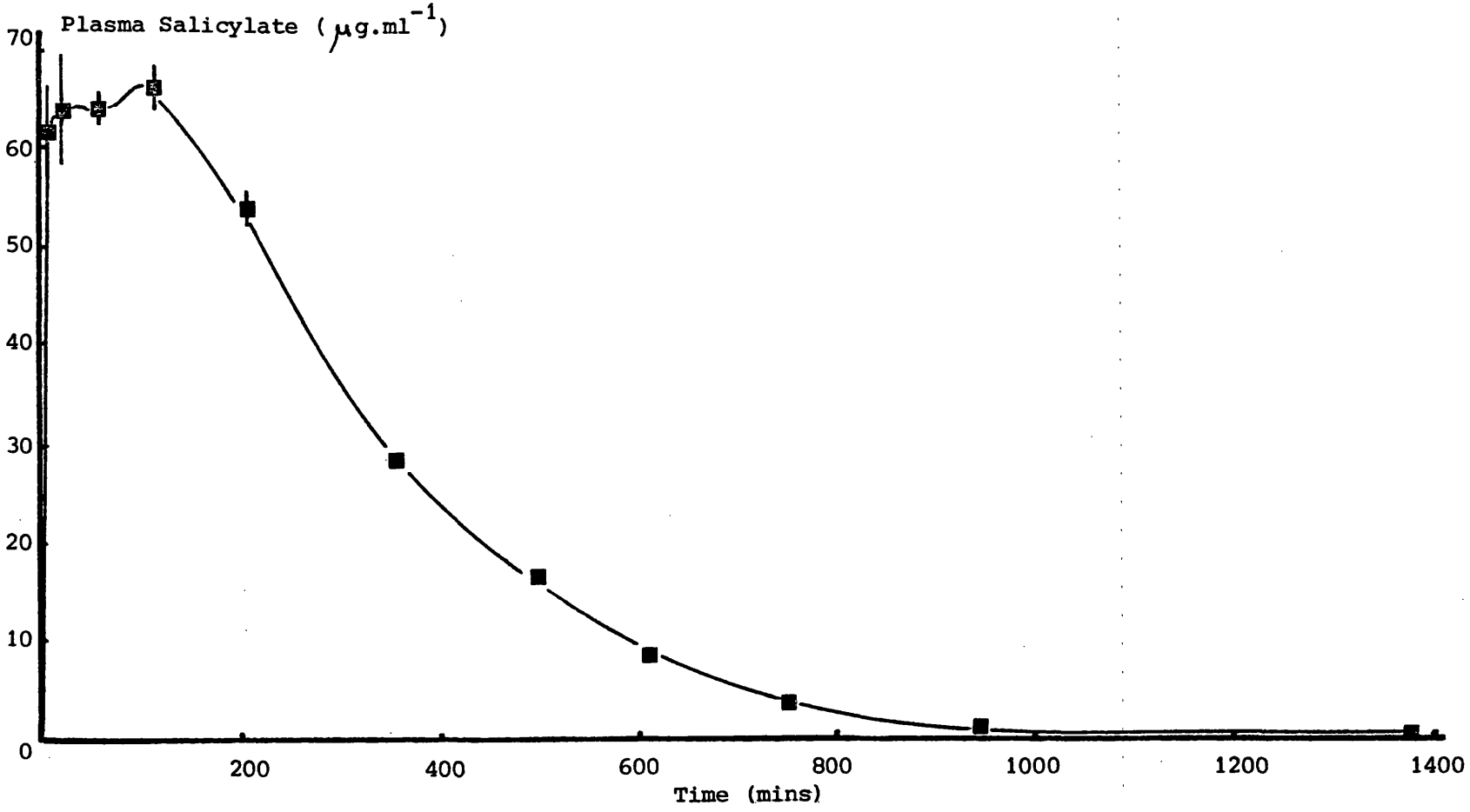


Fig. 3.3.9 Plasma Salicylate Profile For SSA Following Oral Administration In Solution. Dose 20 $mg.kg^{-1}$.

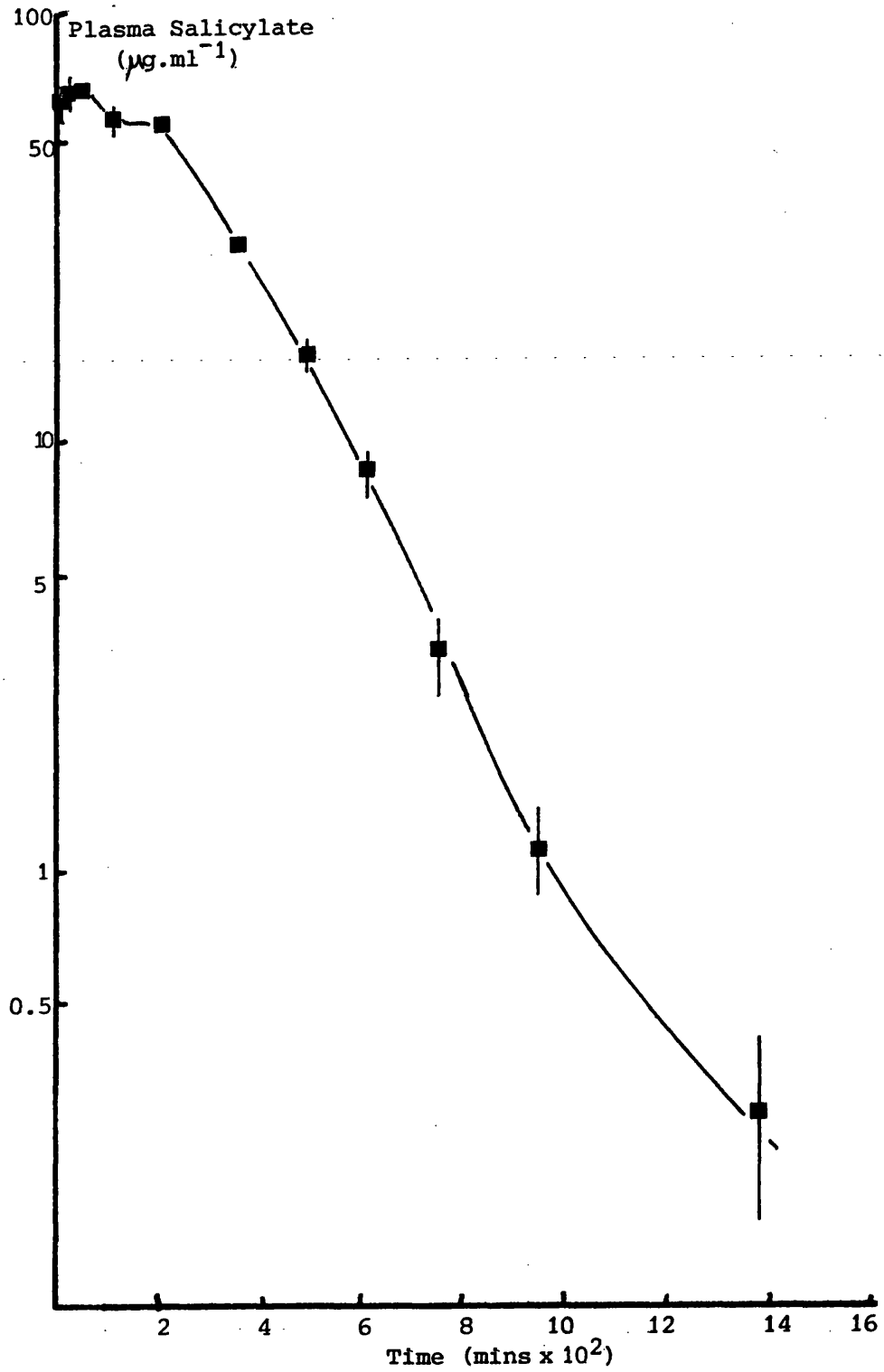


Fig. 3.3.10 Plasma Salicylate (log scale) vs Time Profile For SSA Following Oral Administration In Solution. Dose 20 $mg.kg^{-1}$.

Dissolution studies (Section 2.11) had demonstrated the necessity for a surface active agent to aid wetting and facilitate the exposure of the maximal effective surface area to the dissolution medium. Similarly, initial attempts at preparing SSA suspensions in distilled water, suitable for oral administration to the rat, were abandoned due to incomplete wetting and rapid settling of the material. This was particularly important to avoid in order to ensure that each aliquot of suspended material contained the same percentage of suspended SSA. It was considered that SSA suspensions should, therefore, incorporate a suspending agent that adequately wetted powders of varying particle size producing homogeneous suspensions. Of several potential agents investigated polyvinyl alcohol, (PVA), prepared as a 4% w/v solution, was found to satisfy the above criteria and was therefore used as a vehicle for the oral administration of SSA suspensions. It was of interest then, to examine the possible difference in the absorption of SSA from this vehicle in comparison with that from a simple aqueous suspension.

3.3.11 INFLUENCE OF PVA AS A SUSPENDING AGENT ON SSA ABSORPTION FOLLOWING ORAL ADMINISTRATION

Using a cross-over design with one week between studies, six rats were administered SSA (75-105 μm particle size range; 20 $mg.kg^{-1}$) prepared as a simple aqueous suspension or suspended in 4% w/v PVA. Other experimental conditions were as before (See 3.3.2).

The mean ($\pm$ SE) plasma salicylate *vs* time profiles for both studies, shown in Fig. 3.3.11, indicate a more rapid absorption

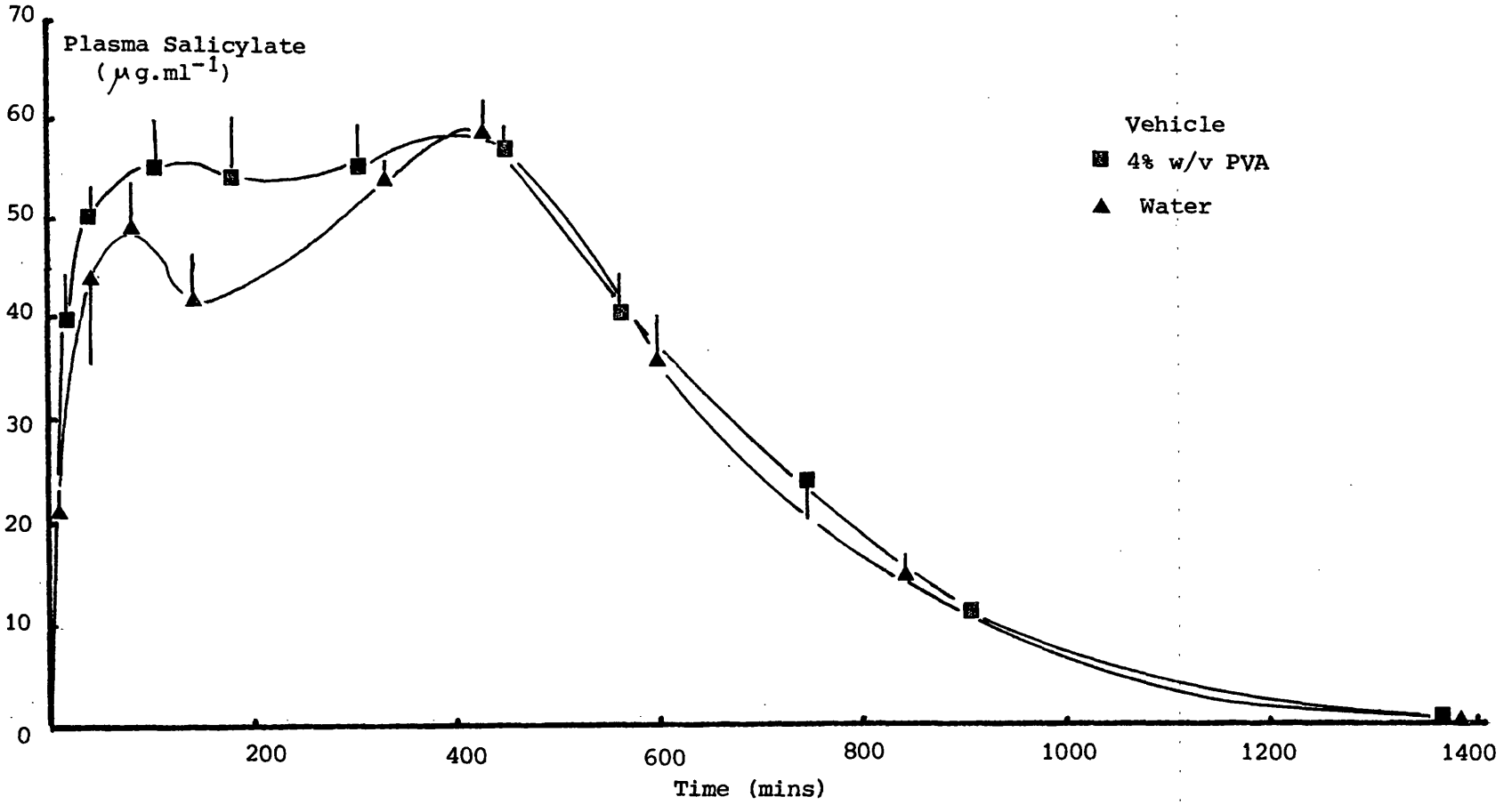


Fig. 3.3.11 Plasma Salicylate Profiles For SSA (75 - 105μm) - Influence Of Vehicle Following Oral Administration. Dose 20 $mg.kg^{-1}$.

from the PVA system than from distilled water. AUC analysis for individual animals is given in Table 3.3.8 with the elimination data for both studies.

The elimination rate constants were calculated from the linear terminal phase of the respective logarithmic profiles given in Fig. 3.3.12 and when compared by the Student 't' test, are shown not to be significantly different (t_{obs} = 1.984; N = 12, p' = 0.05; t_{tab} = 2.228). Similar comparisons between the respective AUC values shows them not to be significantly different either (t_{obs} = 0.982; N = 12; p' = 0.05, t_{tab} = 2.228). The AUC values for animals receiving 20 $mg.kg^{-1}$ SSA as solution (Section 3.3.10) were unexpectedly lower than those for the suspended formulations and will be commented on in later discussion.

Subjective examination of the profiles given in Fig. 3.3.11 shows them both to have a second peak concentration. This is more pronounced in the profile for the water vehicle than that for the PVA vehicle and probably reflects a greater SSA solubility in the PVA solution.

Further discussion regarding secondary peaks will be given later.

3.3.12 INFLUENCE OF DOSE ON PLASMA SALICYLATE LEVELS FOLLOWING ORAL ADMINISTRATION OF SSA SUSPENSIONS IN 4% W/V PVA

The linearity in the relationship between i.v. dose and the area under the curve (AUC) of the resultant plasma salicylate profiles (See Fig. 3.3.8) is indicative that a similar linear dependancy between AUC and dose for oral administration should exist. This

Animal No.	k_{el} ± SE (x $10^{-3}min^{-1}$)		$t_{½}$ (mins)		r		AUC μgml^{-1}x min)	
	4% PVA	Water	4% PVA	Water	4% PVA	Water	4% PVA	Water
1	4.349±0.41	5.550±0.46	159	125	0.996	0.997	50667.1	38336.2
2	5.747±0.82	3.902±0.08	120	178	0.971	0.999	36002.7	35339.2
3	6.767±0.53	5.275±0.74	102	131	0.997	0.990	37880.4	43833.9
4	4.678±0.44	3.459±0.53	148	200	0.996	0.988	41036.1	34710.4
5	4.405±0.91	3.922±0.23	157	177	0.979	0.998	41220.2	36195.2
6	7.174±0.85	3.572±0.53	97	194	0.993	0.989	36387.8	39355.0
mean	5.520	4.280	131	168			40532.4	37961.0
SE	± 0.505	± 0.367	±11	±13			2222.4	1380.0

Table 3.3.8 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$), Correlation Coefficients (r) and Area Under Plasma Level Curve (AUC) Following Oral Administration of SSA as a Suspension in 4% PVA and Distilled Water.

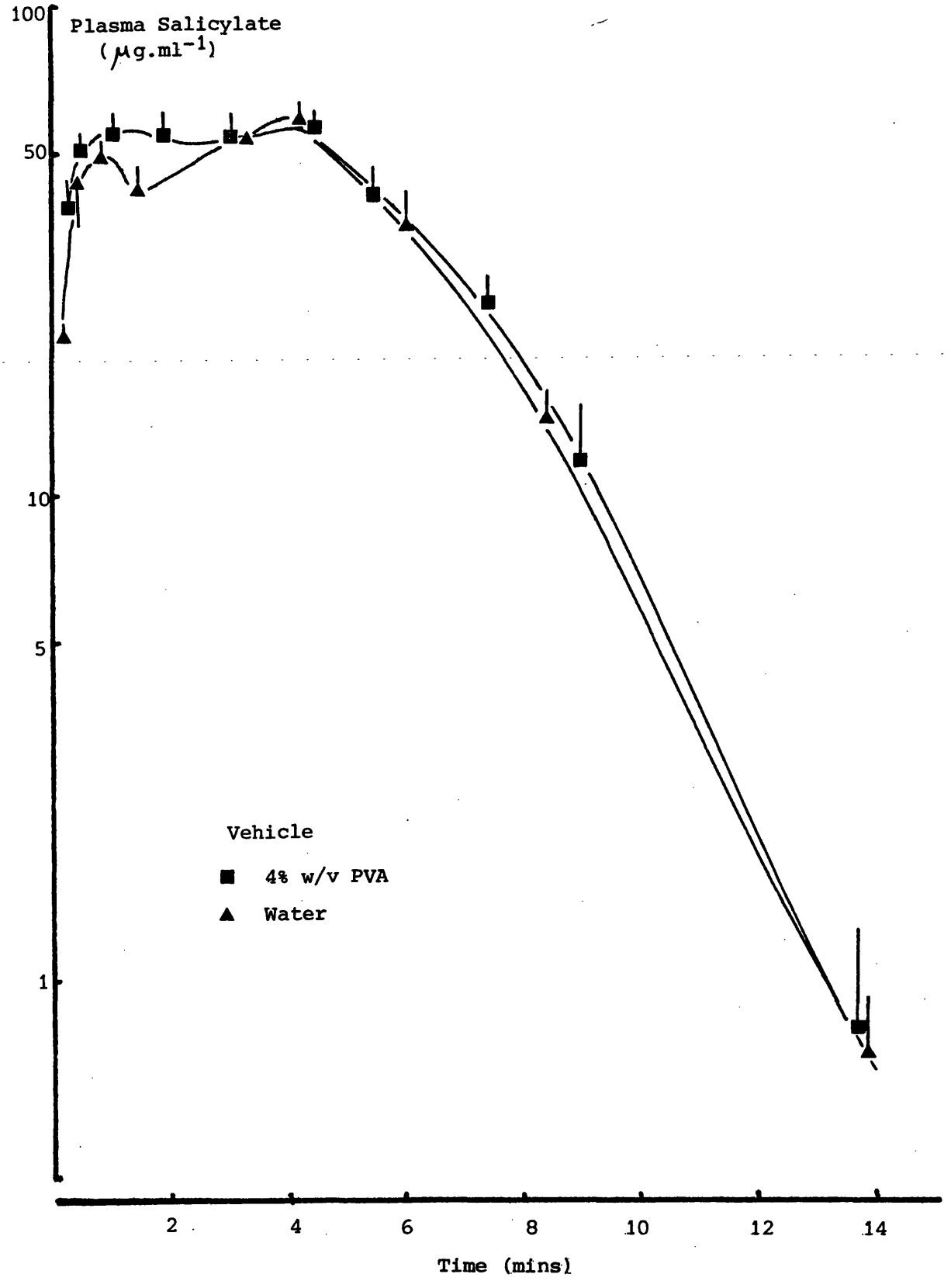


Fig. 3.3.12 Plasma Salicylate (log scale) vs Time Profiles For SSA (75 - 105 μm) - Influence Of Vehicle Following Oral Administration. Dose 20 $mg.kgm^{-1}$.

is based on the assumption that the assay is a determination of total drug plus metabolites. If this relationship does exist it would support the assessment of absorption efficiency by AUC analaysis even in the presence of concomitantly-occurring concentration-dependant and -independant kinetic processes.

SSA (75-105 μm particle size range) prepared as 0.2, 0.4, 1.0 and 2.0% w/v suspensions in 4% w/v PVA, was orally administered by intubation, 0.5 ml 100 g^{-1} body weight, to give doses of 10, 20, 50 and 100 $mg.kg^{-1}$ respectively. Other experimental conditions were as previously described (Section 3.3.2).

The plasma salicylate *vs* time profiles for mean ($\pm$ SE) data, given in Fig. 3.3.13 show a dose-dependant change. Corresponding AUC values for individual animals at each dose level are given in Table 3.3.9 and represented graphically *vs* dose in Fig. 3.3.14, where there is a linear relationship ($r = 0.959$) between dose and AUC for the doses studied.

Apparent first order elimination rate constants (k_{e1}), respective half-lives ($t_{\frac{1}{2}}$) and correlation coefficients (r) are given in Table 3.3.11. The k_{e1} values were calculated following subjective examination of plasma concentration (log scale) *vs* time profiles for individual animals and least-square regression analysis of the appropriate terminal data points. A plot of mean ($\pm$ SE) plasma salicylate concentration (log scale) *vs* time for each dose is given in Fig. 3.3.15. Comparison of the k_{e1} values for each dose is given in Table 3.3.10 where a variance ratio test does not demonstrate any significant difference between the overall

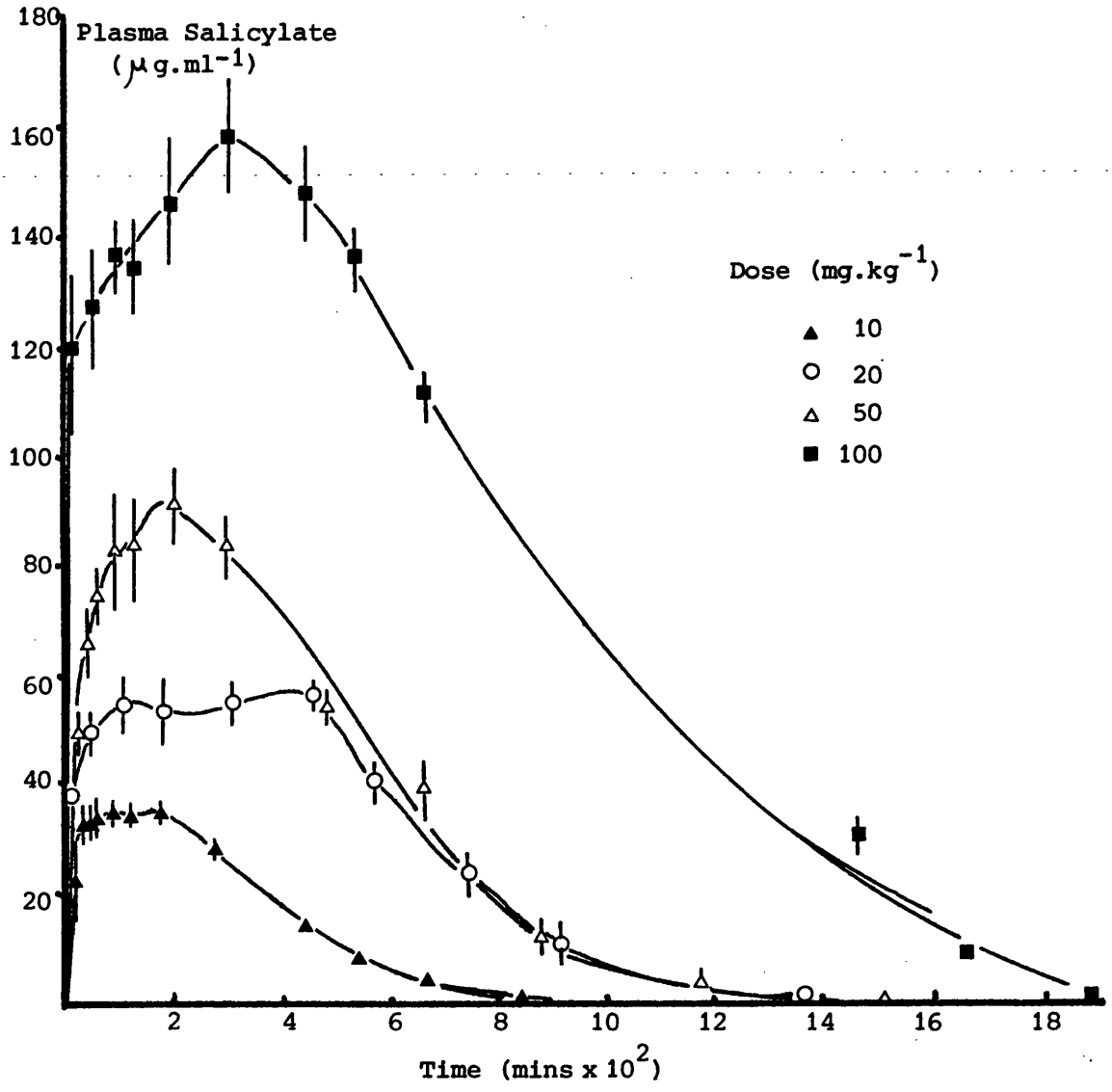


Fig. 3.3.13 Plasma Salicylate Profiles For SSA (75 - 105μm) - Influence Of Dose Following Oral Administration.

Animal	Dose ($mg.kg.^{-1}$)			
	100	50	20	10
1	166141	48525	50667	16750
2	165899	48510	36003	14144
3	146081	54792	37880	14416
4	156040	37711	41036	13791
5	129965	54370	41220	12635
6	167122	64133	36388	13992
Mean	155208	51340	40532	14288
+ SE	6047	3589	2222	553

Table 3.3.9 Influence Of Dose On AUC ($\mu g.\ ml.^{-1} x\ min$) Following Oral Administration of SSA, 75-105 μm Particle Size In A 4% w/v PVA Suspension

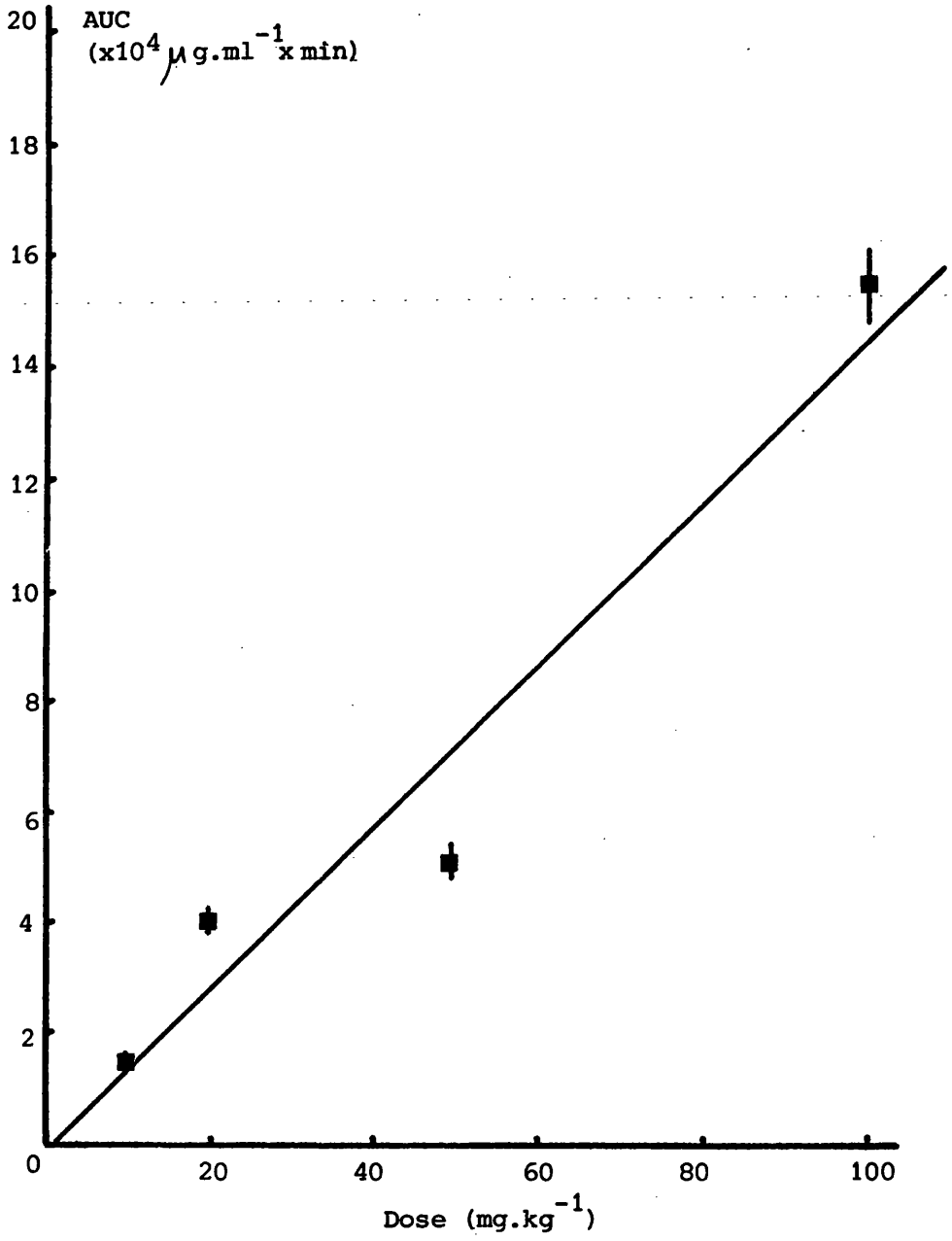


Fig. 3.3.14 Relationship Between Area Under The Plasma Level Curve (AUC) And Dose For SSA (75 - 105 μm) Following Oral Administration.

Dose ($mg.kg^{-1}$)		1	2	3	4	5	6	Mean $\pm$ SE
100	$k_{el} \pm$ SE ($x10^{-3}min^{-1}$)	-	6.758 $\pm$0.17	-	5.300 $\pm$1.08	4.727 $\pm$0.87	4.711 $\pm$0.66	5.374 $\pm$0.096
	$t_{½}$ (mins)	-	103	-	131	147	147	132 $\pm$ 10
	r	-	0.999	-	0.979	0.983	0.990	-
50	$k_{el} \pm$ SE ($x10^{-3}min^{-1}$)	8.626 $\pm$1.40	5.239 $\pm$0.31	5.179 $\pm$0.85	5.159 $\pm$0.43	8.726 $\pm$0.55	6.403 $\pm$0.85	6.555 $\pm$0.506
	$t_{½}$ (mins)	80	132	134	134	79	108	112 $\pm$ 11
	r	0.987	0.997	0.962	0.993	0.998	0.991	-
20	$k_{el} \pm$ SE ($x10^{-3}min^{-1}$)	4.349 $\pm$0.41	5.747 $\pm$0.82	6.767 $\pm$0.53	4.678 $\pm$0.44	4.405 $\pm$0.91	7.174 $\pm$0.85	5.520 $\pm$0.505
	$t_{½}$ (mins)	159	121	102	148	157	97	131 $\pm$ 11
	r	0.996	0.971	0.997	0.996	0.979	0.993	-
10	$k_{el} \pm$ SE ($x10^{-3}min^{-1}$)	4.933 $\pm$0.15	8.598 $\pm$0.37	5.760 $\pm$1.94	5.630 $\pm$0.17	4.442 $\pm$1.16	6.786 $\pm$1.35	6.025 $\pm$0.090
	$t_{½}$ (mins)	140	81	119	123	156	102	120 $\pm$ 11
	r	0.999	0.998	0.948	0.999	0.968	0.963	-

Table 3.3.11 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA, 75-105 μm At Four Dosage Levels.

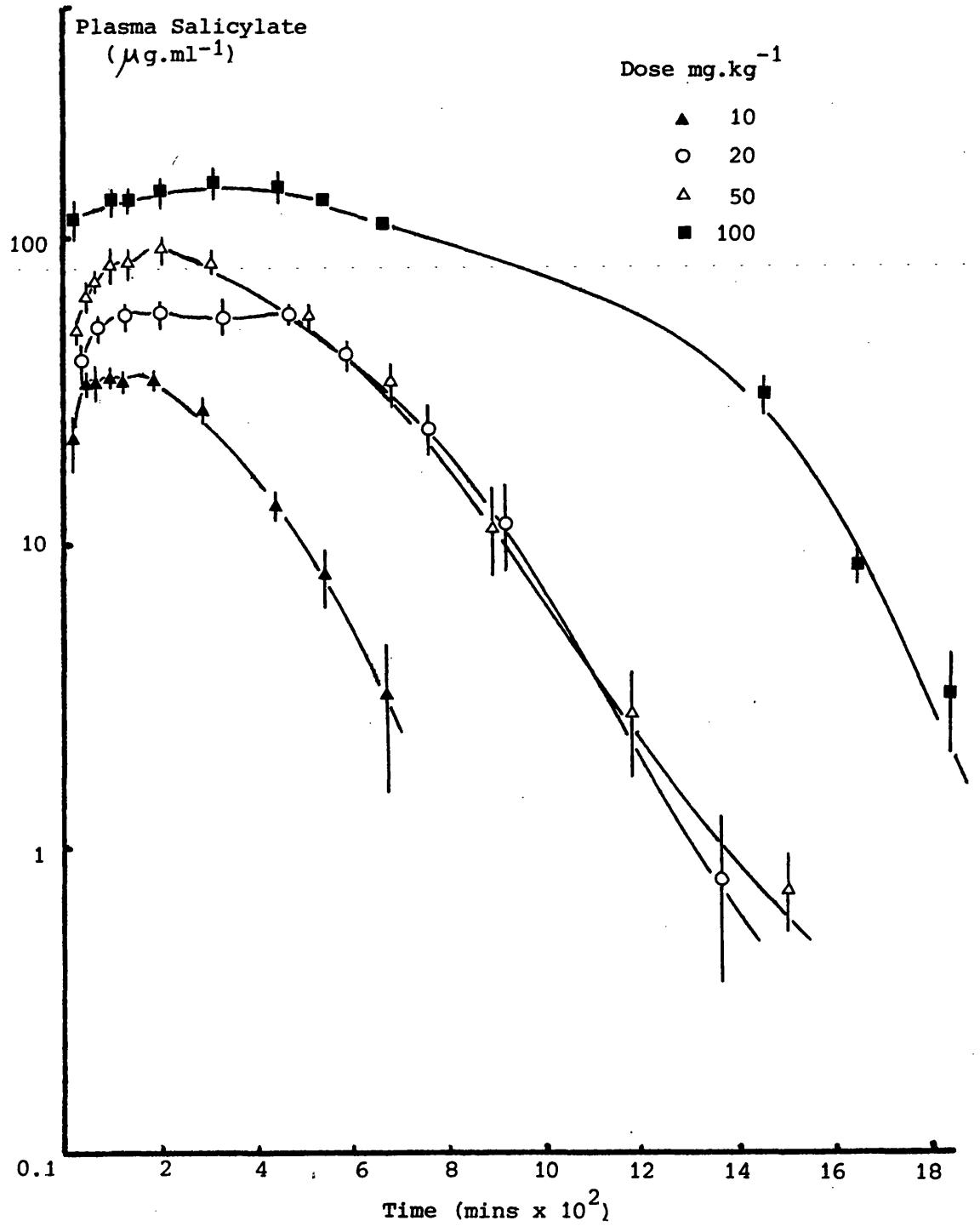


Fig. 3.3.15. Plasma Salicylate (log scale) vs Time Profiles For SSA (75 - 105 μm) - Influence Of Dose Following Oral Administration.

rate constants for salicylate elimination.

Source of Variation	Sum Sq.	Degrees of Freedom	Mean Sq.	F Value	F Ratio	F Tabulated
K_{el}	5.462	3	1.821	0.758	1.831	$F_5^3=5.41$
Animal	4.973	5	0.995	0.414		p'=0.05
Residual	3.122	13	2.402			
Total	4.166	21				

Table 3.3.10 Variance Ratio Test for SSA Following Oral Administration at Four Dosage Levels - Elimination.

It can therefore be expected that changes in the extent of SSA absorption from suspended experimental dosage forms would be reflected by changes in the AUC even though capacity-limited processes are known to occur.

3.3.13 INFLUENCE OF SSA PARTICLE SIZE ON PLASMA SALICYLATE LEVELS FOLLOWING ORAL ADMINISTRATION

The correlation between BET surface area and dissolution rate for various particle size ranges of SSA has already been demonstrated (Section 2.11, Fig. 2.11.4). This *in vitro* relationship may not necessarily be reflected by the *in vivo* absorption of SSA from suspension unless absorption is dissolution rate-limited. To establish whether or not SSA absorption is a function of dissolution rate various particle size fractions of the drug were administered *per os* and the

resultant plasma salicylate concentration *vs* time profiles examined.

Various particle size ranges were formulated as 0.4% w/v suspensions in 4% w/v PVA and orally administered by intubation, 0.5 ml 100 g^{-1} body weight, to give a dose of 20 $mg.kg^{-1}$.

The mean ($\pm$ SE) plasma salicylate profiles for the six ranges of particle size are shown in Figs. 3.3.16 and 3.3.17. Parameters such as peak times and the occurrence of a secondary peak will be discussed later in an attempt to correlate them with drug availability and the *in vitro* dissolution data previously presented. Respective AUC data is given in Table 3.3.12 and compared by a variance ratio test in Table 3.3.17.

Apparent first order elimination rate constants, calculated from the terminal linear phase of a first order plot for each animal, are given in Tables 3.3.13 and 3.3.14. First order plots for mean ($\pm$ SE) plasma salicylate concentration *vs* time profiles are shown in Figs. 3.3.18 and 3.3.19. A variance ratio test for possible particle size effects on salicylate elimination was carried out and although the result (Table 3.3.17) does not show any significant effect the variance due to formulation was greater than that due to biological variation. The k_{el} values for each particle size were subsequently compared by a Student 't' test (Table 3.3.15) which shows that elimination following oral dosing with the 150-180 μm particle size material was significantly slower than all other particle size fractions.

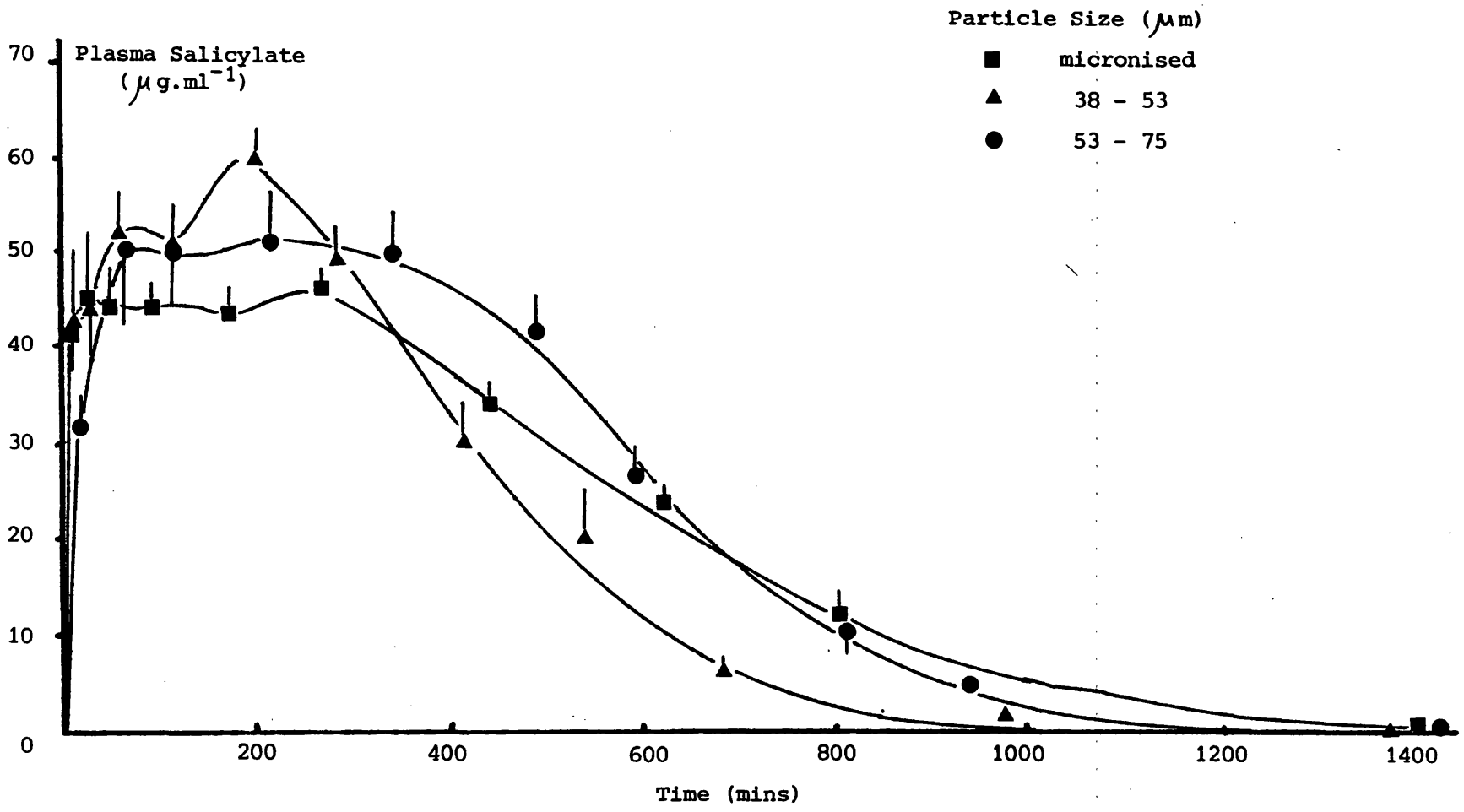


Fig. 3.3.16 Plasma Salicylate Profiles For SSA - Influence Of Particle Size. Dose 20 $mg.kg^{-1}$

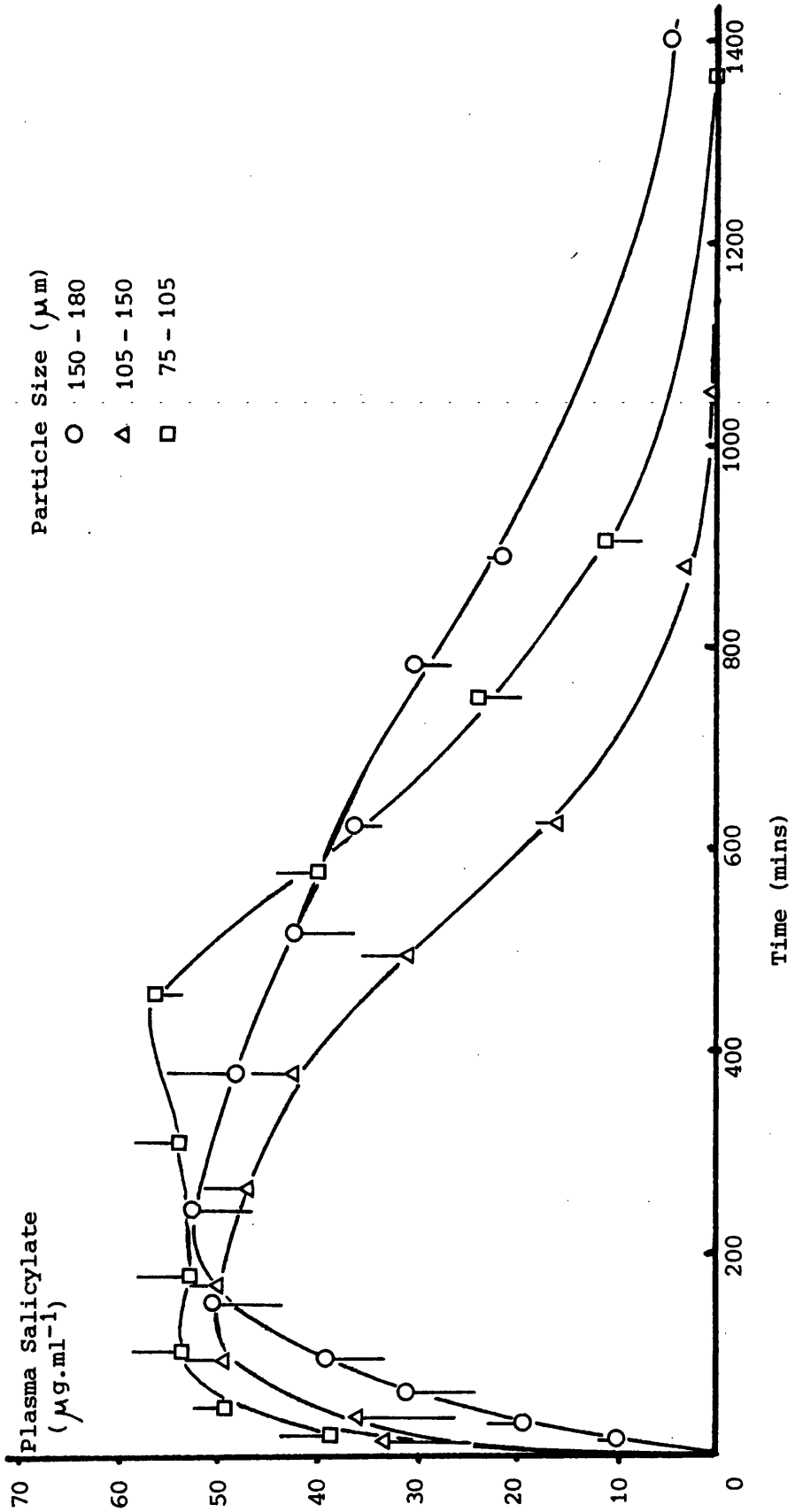


Fig. 3.3.17 Plasma Salicylate Profiles For SSA - Influence Of Particle Size. Dose 20 $mg.kg^{-1}$.

AUC ($\mu g.\ ml.^{-1} \times min$) For Particle Size Ranges (μm)

Micron	38-53	53-75	75-105	105-150	150-180
29456	22952	44278	50667	29842	39558
40848	30307	28258	36003	24320	56864
27375	22220	27336	37880	30808	37716
34917	31649	32841	41036	29371	41564
29277	-	36708	41220	24920	34928
27745	25518	30463	36388	23363	46765
Mean 31603	26529	33314	40532	27104	42859
± SE 2154	1909	2590	2222	1328	3232

Table 3.3.12 Area Under Plasma Salicylate Profile Following Oral Administration of SSA Of Varying Particle Size. Dose 20mg kg^{-1}

		1	2	3	4	5	6	mean
micronized	$k_{el} \pm SE$ ($x10^{-3}min^{-1}$)	4.382 ±0.30	3.892 ±0.41	- -	3.010 ±0.19	4.553 ±0.12	5.358 ±0.53	4.239 ±0.387
	$t_{½}$ (mins)	158	178	-	230	152	129	170±17
	r	0.995	0.995	-	0.992	0.999	0.990	-
38 - 53µm	$k_{el} \pm SE$ ($x10^{-3}min^{-1}$)	10.327 ±0.26	5.437 ±0.64	3.979 ±0.31	8.575 ±0.30	4.041 ±0.15	- -	6.472 ±1.274
	$t_{½}$ (mins)	67	128	174	81	172	-	124±22
	r	0.999	0.987	0.994	0.999	0.999	-	-
53 - 75µm	$k_{el} \pm SE$ ($x10^{-3}min^{-1}$)	4.261 ±0.05	3.828 ±0.27	6.409 ±0.42	7.189 ±1.18	4.797 ±0.23	4.150 ±0.41	5.106 ±0.354
	$t_{½}$ (mins)	163	181	108	96	144	167	143±14
	r	0.999	0.993	0.994	0.974	0.998	0.986	-

Table 3.3.13 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA of Micronized, 38-53 and 53-75µm Particle Size in Suspension with 4% PVA.

		1	2	3	4	5	6	mean
75-105 μm	k_{el} ± SE ($x10^{-3}min^{-1}$)	4.349 ±0.41	5.747 ±0.82	6.767 ±0.53	4.678 ±0.44	4.405 ±0.91	7.174 ±0.85	5.520 ±0.505
	$t_{½}$ (mins)	159	121	102	148	157	97	131±11
	r	0.996	0.971	0.997	0.996	0.979	0.993	-
105-150 μm	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.280 ±0.18	5.185 ±0.54	5.281 ±0.50	7.801 ±0.32	6.237 ±0.53	6.096 ±0.38	5.980 ±0.409
	$t_{½}$ (mins)	131	133	132	89	111	114	118±7
	r	0.998	0.984	0.987	0.997	0.996	0.994	
150-180 μm	k_{el} ± SE ($x10^{-3}min^{-1}$)	3.074 ±0.29	3.2125 ±0.17	- -	3.710 ±1.30	2.911 ±0.14	- -	3.227 ±0.172
	$t_{½}$ (mins)	223	216	-	187	238	-	217±11
	r	0.983	0.999	-	0.943	0.998	-	-

Table 3.3.14 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA of 75-105, 105-150, 150-180 μm Particle Size in Suspension with 4% PVA.

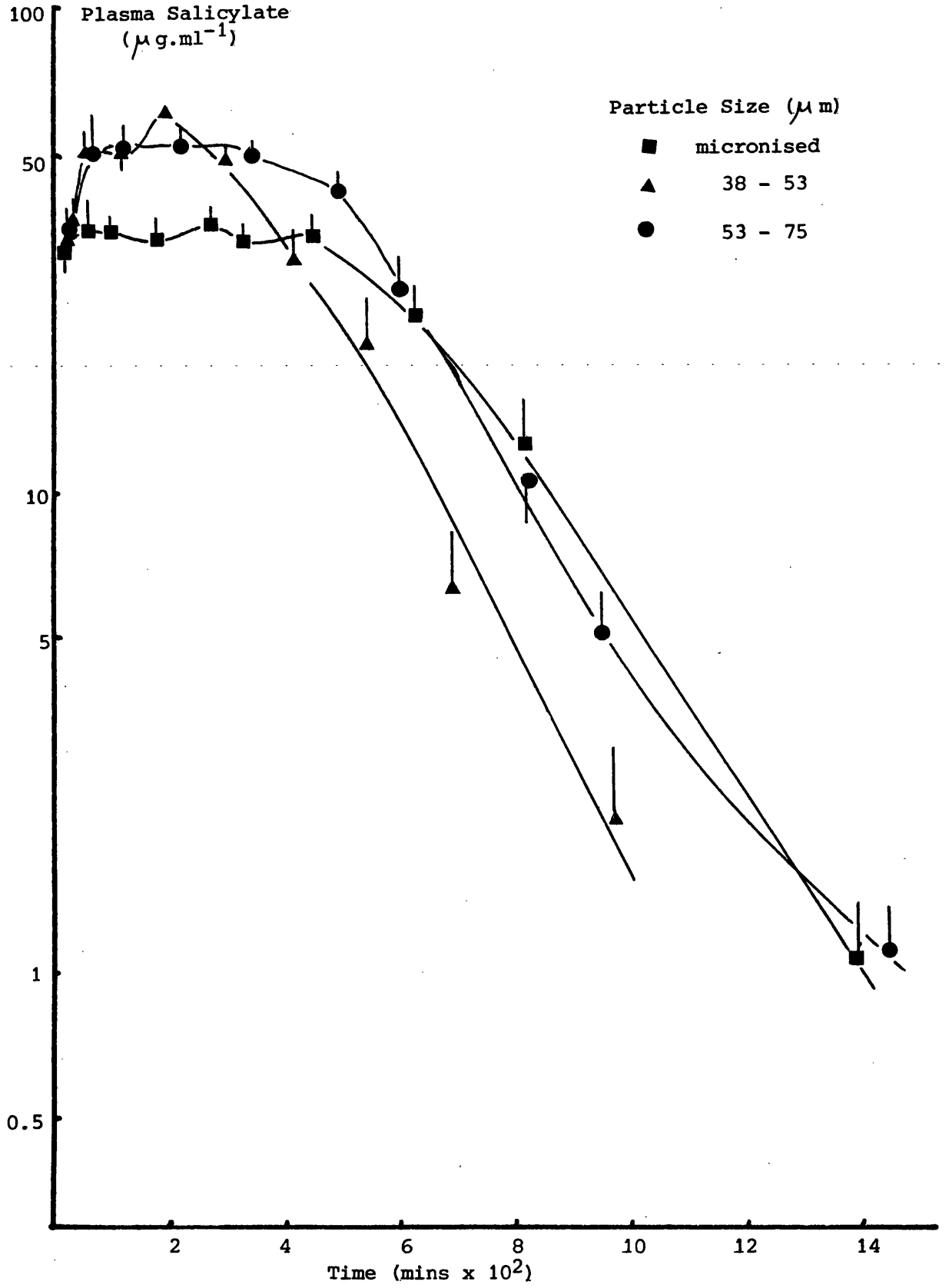


Fig. 3.3.18 Plasma Salicylate (log scale) vs Time Profiles For SSA - Influence Of Particle Size. Dose 20 $mg.kg^{-1}$.

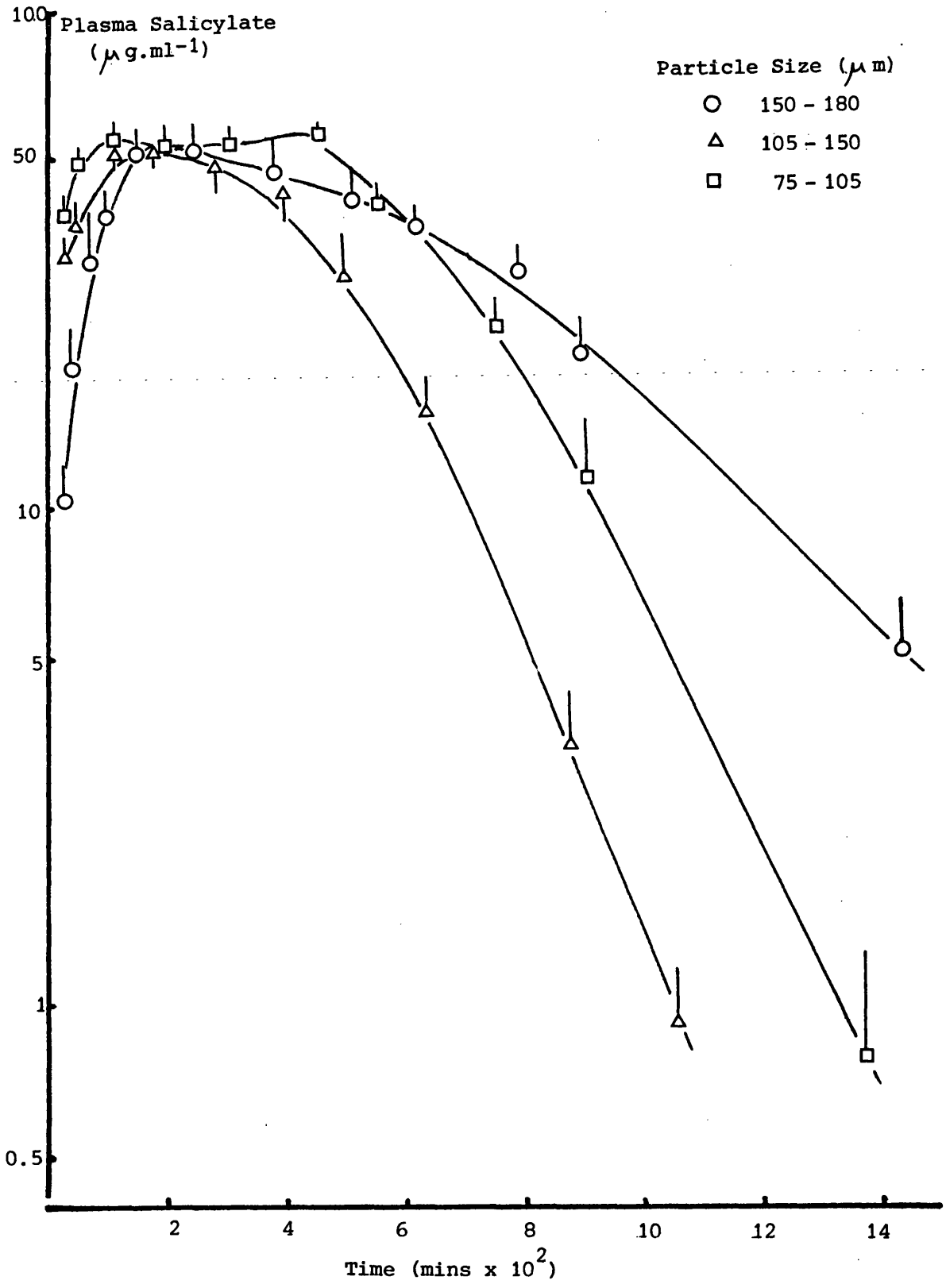


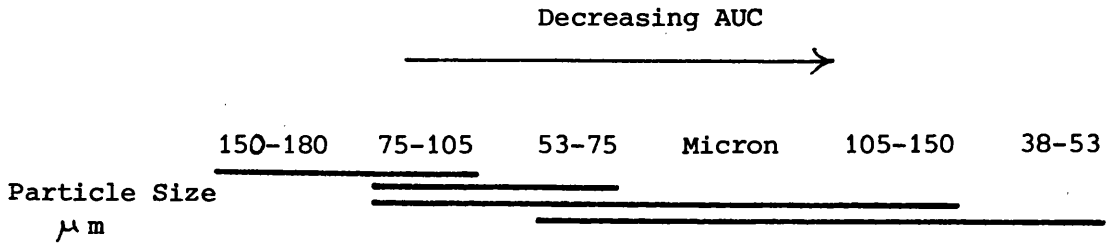
Fig. 3.3.19 Plasma Salicylate (log scale) vs Time Profiles For SSA - Influence Of Particle Size. Dose 20 $mg.kg^{-1}$.

Particle Size (μm)	'Micron'	38-53	53-75	75-105	105-150
38-53	1.676 N = 10				
53-75	1.273 N = 11	0.982 N = 11			
75-105	2.011 N = 11	0.694 N = 11	0.550 N = 12		
105-150	3.092 N = 11	0.368 N = 11	1.262 N = 12	0.708 N = 12	
150-180	2.386 N = 9	2.523 N = 9	3.209 N = 10	4.293 N = 10	6.209 N = 10

For N = 9 $t_{tab} = 2.365$
N = 10 $t_{tab} = 2.306$
N = 11 $t_{tab} = 2.262$
N = 12 $t_{tab} = 2.228$
} $p' = 0.05$

Table 3.3.15 Student 't' Test Comparison Between Apparent First Order Elimination Rate Constants (K_{el}) for SSA of Various Particle Size Ranges Following Oral Administration 20 $mg.kg.^{-1}$

Since the variance ratio test for the AUC data has shown particle size to be an influential factor, a Student 't' test comparison between the values for each formulation was performed and is shown in Table 3.3.16. The results of this test are depicted below, where the particle size ranges are given in order of decreasing AUC, and the bars show where no significant difference exists between the particle size fractions (at the 5% confidence level).



These results will be further discussed in Chapter 4.

The variance ratio analysis for both the elimination and AUC data given in Table 3.3.17 shows there to be a significant effect of particle size on the AUC in comparison to that of animal origin. While indicating particle size to be influential on the AUC it does not follow that the variances for AUC and k_{e1} are related even though a change in k_{e1} will produce a corresponding change in AUC (assuming all other pharmacokinetic functions remain unchanged). Examination of the salicylate profiles of the mean data for each particle size range show obvious alterations in the attainment of peak levels and the concentrations themselves, factors which suggest a change in the rate and extent of absorption. Consequently the particle size related variation in

Particle Size (μm)	'Micron'	38-53	53-75	75-105	105-150
38-53	1.763 N = 11				
53-75	0.508 N = 12	2.106 N = 11			
75-105	2.885 N = 12	4.780 N = 11	2.115 N = 12		
105-150	1.778 N = 12	0.247 N = 11	2.133 N = 12	5.188 N = 12	
150-180	2.905 N = 12	4.362 N = 11	2.314 N = 12	0.603 N = 12	4.521 N = 12

For N = 12 $t_{tab} = 2.228$
For N = 11 $t_{tab} = 2.262$
} p' = 0.05

Table 3.3.16 Student 't' Test Comparison Between AUC Values for SSA of Various Particle Size Ranges Following Oral Administration, 20 $mg.kg.^{-1}$

	Source of variation	Sum Sq	Degrees of Freedom	Mean Sq	F Value	F Ratio	F tabulated
Elimination Rate	K_{el}	42.79	5	8.557	3.635	4.65	F_5^5 = 5.05 p'=0.05
	Animal	9.12	5	1.827	0.775		
	Residual	49.42	21	2.354			
	Total	101.3	31				
AUC ($x10^{-8}$)	AUC	14.31	5	2.813	9.163	7.10	F_5^5 = 5.05 p'=0.05
	Animal	1.980	5	0.396	1.290		
	Residual	7.379	24	0.307			
	Total	23.69	34				

Table 3.3.17 Variance Ratio Test For SSA Following Oral Administration Of Varying Particle Sizes - Elimination And AUC.

AUC cannot be associated with the changes in k_{e1} *per se*.

The significance and possible explanation for these findings will be discussed later.

Urinary excretion studies with SSA in man (Section 3.2.3) have shown urinary excretion data to be unable to adequately discriminate between the influences of particle size on SSA absorption. To establish whether the same criticism can be levied for similar studies in the rat the following investigations were carried out.

3.3.14 INFLUENCE OF PARTICLE SIZE ON SSA ABSORPTION FROM SUSPENSION AS MEASURED BY URINARY EXCRETION OF TOTAL SALICYLATE

1% w/v aqueous suspensions of various particle size ranges of SSA were prepared in 4% w/v PVA. Animals received 1 ml 100 g^{-1} body weight (100 $mg.kg^{-1}$) and urine collected 4 and 24 hours after dosing. The mean ($\pm$ SE) % dose excreted after these times are given for each particle size range in Table 3.3.18.

To examine for particle size related changes in % dose excreted a variance ratio test was performed and the results given in Table 3.3.19 for both time samples.

Particle Size (μm)	Mean % Dose Excreted (± SE) After:	
	4 hours	24 hours
150 - 180	21.09 ± 0.82	48.20 ± 5.23
105 - 150	13.40 ± 2.36	59.62 ± 3.10
75 - 105	16.09 ± 2.66	68.99 ± 1.39
53 - 75	12.87 ± 2.51	57.97 ± 2.21
38 - 53	18.16 ± 1.43	64.07 ± 4.59
30 - 38	17.38 ± 2.51	56.62 ± 1.77
Micronised	19.60 ± 2.85	58.51 ± 3.36
Vehicle Control	0.05 ± 0.01	0.16 ± 0.01

Table 3.3.18 Dose SSA Excreted After 4 and 24 Hours Following Oral Administration of SSA Suspensions of Various Particle Sizes.

It is evident that the variance in % dose excreted associated with particle size is not significantly greater than that associated with animal variation for either the 4 or 24 hour samples, however, although urinary excretion of total salicylate has shown no significant variation that could be attributed to particle size after 24 hours, there was a suggestion that after 4 hours some influence was evident (ie higher F value, Table 3.3.19). If coprecipitation of SSA with PVP or the bile acids had any significant influence of absorption then it may be reflected in the urinary excretion of total salicylate. To examine this possibility absorption of SSA from SSA:PVP coprecipitates was estimated by measurement of total salicylate

Time	Source of variation	Sum Sq	Degrees of	Mean Sq	F value	F ratio	F tabulated
4 hours	Particle Size	577.91	6	96.318	2.174	3.70	F_5^6 = 4.95 p'=0.05
	Animal	130.09	5	26.017	0.587		
	Residual	1329.14	30	44.305			
	Total	2037.14	41				
24 hours	Particle Size	870.19	6	145.03	1.618	1.06	F_5^6 = 4.95 p'=0.05
	Animal	686.38	5	137.28	1.532		
	Residual	2688.94	30	89.63			
	Total	4245.30	41				

Table 3.3.19. Variance Ratio Test For % Dose Excreted 4 And 24 hours Following Oral Administration of SSA of Various Particle Sizes

in the urine 4, 24 and 48 hours after oral dosing.

3.3.15 INFLUENCE OF COPRECIPITATION OF SSA WITH PVP ON SSA ABSORPTION FROM SUSPENSION BY MEASUREMENT OF URINARY EXCRETION OF TOTAL SALICYLATE

SSA:PVP coprecipitates were formulated as 1% w/v (equivalent SSA) suspensions in 4% w/v PVA using 75-105 μm material. Each ratio was adjusted for SSA content according to their % SSA values reported previously (See 2.13.1.1). Groups of six animals were orally-dosed with these suspensions at 100 mg (equivalent SSA)kg^{-1}, urine was collected after 4, 24 and 48 hours and assayed as described.

The total salicylate excreted, expressed as mean ($\pm$ SE) % dose is given in Table 3.3.20 for the SSA:PVP (2:1, 1:1, 1:2 and 1:5) coprecipitates.

Coprecipitate SSA:PVP ratio	Mean $\pm$ SE % Dose Excreted After:		
	4 hours	24 hours	48 hours
2:1	11.90 $\pm$ 4.40	70.53 $\pm$ 8.31	74.99 $\pm$ 8.32
1:1	15.63 $\pm$ 2.36	71.38 $\pm$ 2.82	76.14 $\pm$ 2.18
1:2	14.37 $\pm$ 2.87	63.90 $\pm$ 4.20	73.79 $\pm$ 5.89
1:5	7.10 $\pm$ 1.61	34.16 $\pm$ 2.79	47.27 $\pm$ 2.83

Table 3.3.20 Urinary Excretion of Total Salicylate Following Oral Administration of SSA:PVP Coprecipitates.

Students 't' test comparison between the % dose excreted for the SSA:PVP coprecipitates with SSA alone (data taken from 75-105 μm

particle size study given in Table 3.3.18) after 4 and 24 hours shows that the 1:5 ratio significantly retarded the efficiency of SSA absorption. ('t' values of 2.893 and 11.179 were calculated for the 4 and 24 hour samples respectively). The other ratios did not show any influence that was statistically distinguishable from SSA alone for either the 4 or 24 hour data. The data for all comparisons are given in Table 3.3.21.

Coprecipitate SSA:PVP Ratio	Students 't' value for 4 hours	Students 't' value for 24 hours	't' tabulated
1:5	2.893	11.179	In all cases
1:2	0.440	1.152	$t_{tab} = 2.228$
1:1	0.136	0.759	$p' = 0.05$
2:1	0.861	0.194	$N = 12$

Table 3.3.21 Student 't' Test Between % Dose Excreted After 4 and 24 Hours Following Oral Administration of SSA:PVP Coprecipitates In Comparison To SSA Alone.

It is evident that the 't' values for the 24 hour data in Table 3.3.21 above decrease with PVP content of the coprecipitate. The significance of this will be discussed in due course. A Student 't' test comparison between the % dose excreted for all three time intervals is given in Table 3.3.22 for the SSA:PVP coprecipitates where it can be seen that the % dose excreted following administration of the 1:5 ratio is significantly different from the other SSA:PVP ratios at all times, with the exception of the four hour excretion for the 2:1 ratio. Coprecipitates other than the 1:5 ratio show no significant

% Dose Excreted After	SSA:PVP Ratio	SSA:PVP RATIO 1:5	1:2	1:1
4 hours	1:2	2.215 N = 11		
	1:1	2.983 N = 12	0.336 N = 11	
	2:1	1.026 N = 11	0.471 N = 10	0.745 N = 11
24 hours	1:2	5.903 N = 11		
	1:1	9.391 N = 12	1.479 N = 11	
	2:1	4.160 N = 11	0.723 N = 10	0.085 N = 11
48 hours	1:2	4.057 N = 11		
	1:1	8.091 N = 12	0.374 N = 11	
	2:1	3.154 N = 11	0.118 N = 10	0.134 N = 11

For N = 10 t_{tab} = 2.306
N = 11 t_{tab} = 2.262 p' = 0.05 df = N - 2
N = 12 t_{tab} = 2.228

Table 3.3.22 Students 't' Test Between % Dose Excreted After 4, 24 and 48 Hours Following Oral Administration of SSA:PVP 1:5, 1:2, 1:1 and 2:1 Coprecipitates.

differences. These results indicate that the extent of SSA absorption from the 1:5 SSA:PVP coprecipitate is significantly retarded in comparison to the other coprecipitates and to SSA alone.

As suggested in the human studies, and in previous studies in the rat, the urinary excretion method of discriminating between SSA formulations was only able to differentiate between gross changes in bioavailability.

To follow the appearance of salicylate in the plasma would therefore provide a more precise and valid method of assessing the bioavailability of SSA from its coprecipitates and admixtures with PVP, cholic acid and deoxycholic acid.

3.3.16 ABSORPTION OF SALICYLATE FROM SSA:PVP COPRECIPITATES AND ADMIXTURES

The general experimental protocol has been described (See 3.3.2). All animals received 20 mg (equivalent SSA) kg^{-1} body weight by oral intubation, each dosage being corrected for % SSA content using the values given in Table 2.13.4. Dosage of the admixtures was corrected for % SSA content according to the ratio of the components in the physical mix. Blood samples, taken at zero time and at appropriate time intervals following dosing were assayed for salicylate as described (See 3.3.7).

The mean ($\pm$ SE) plasma salicylate concentration *vs* time profiles are shown in Figs. 3.3.20 to 3.3.24 for the 2:1, 1:1, 1:2, 1:5 and 1:10 coprecipitates and admixtures respectively. Peak

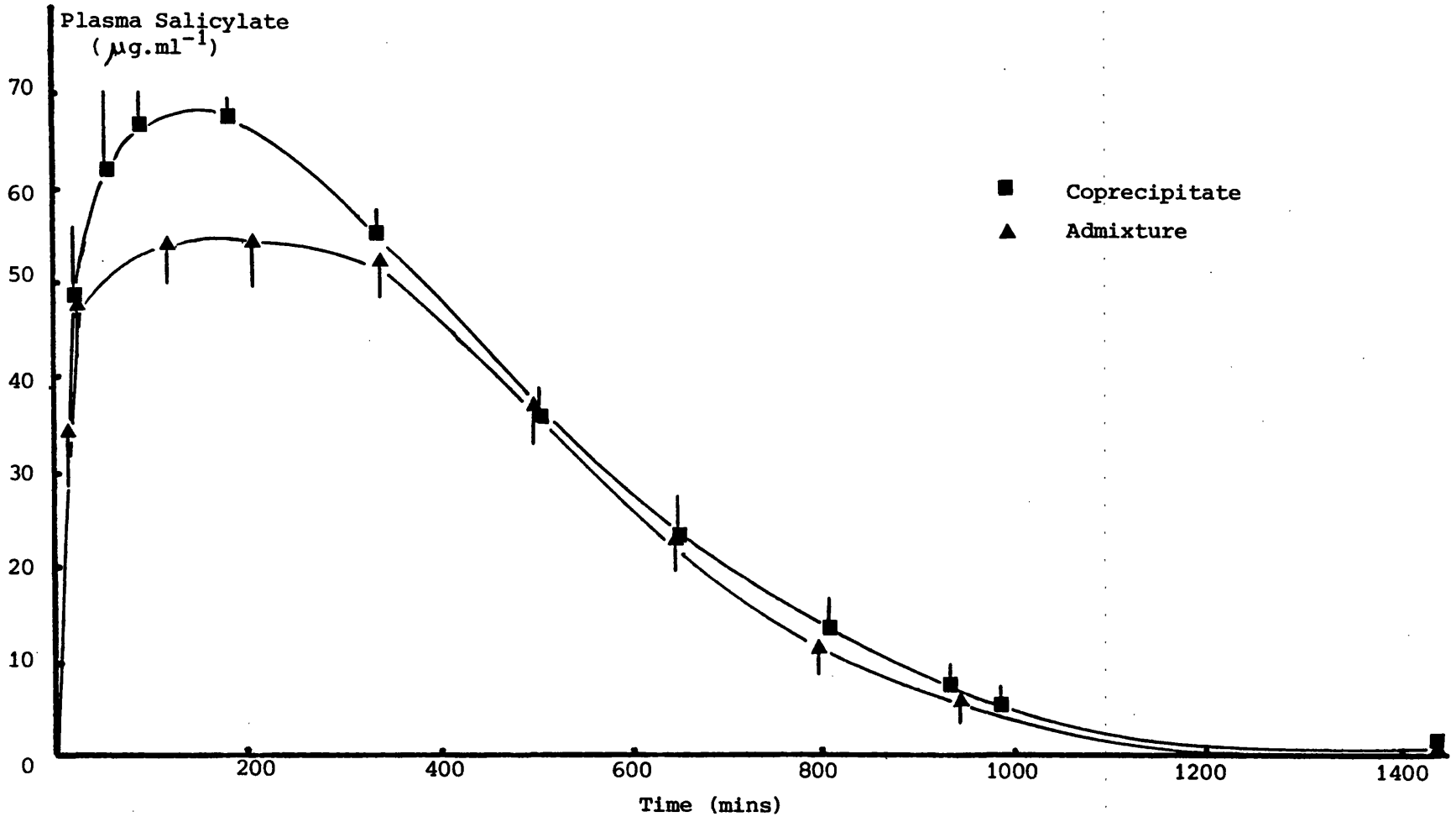


Fig. 3.3.20 Plasma Salicylate Profiles For SSA:PVP 2:1 Coprecipitate And Admixture

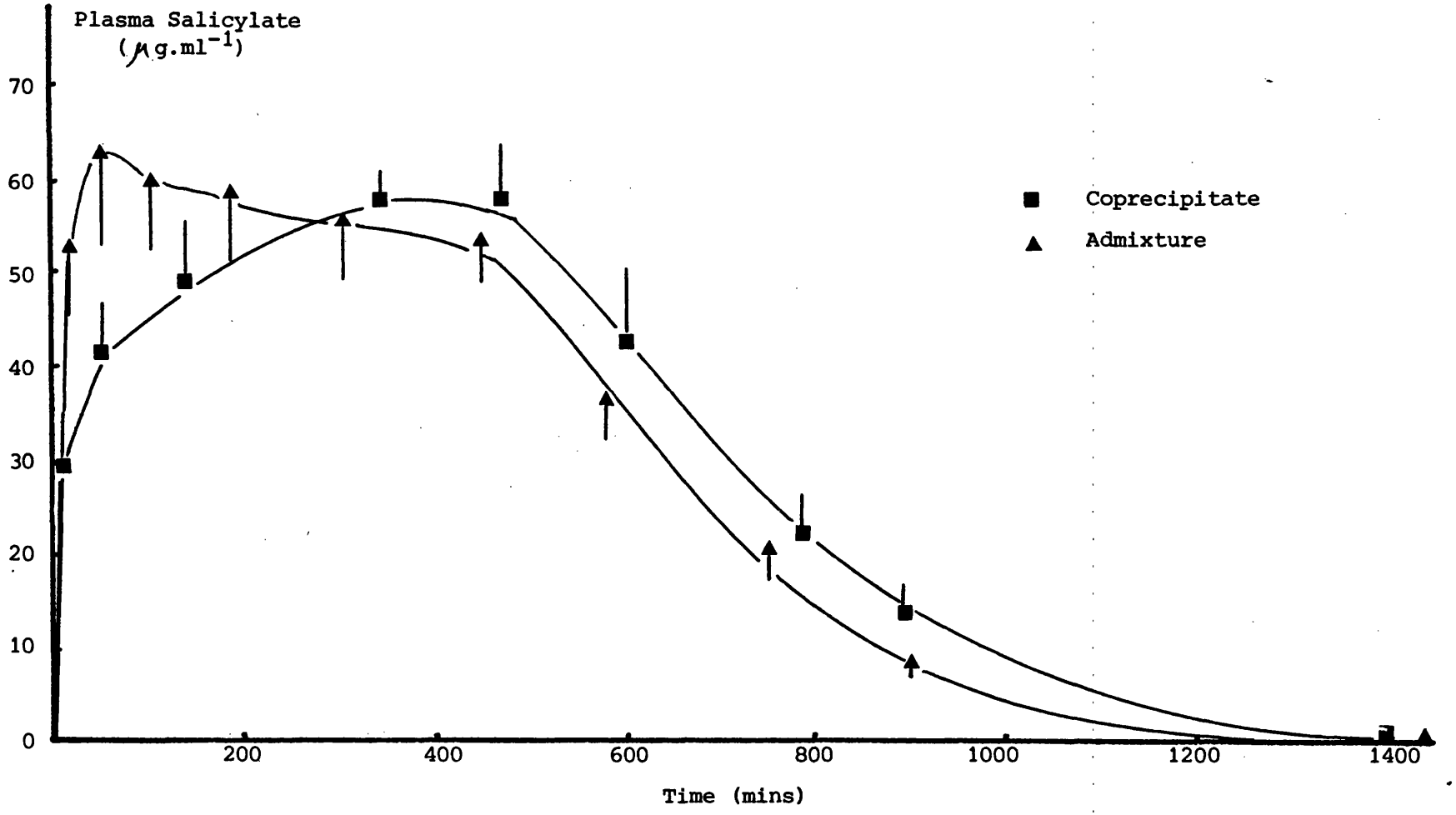


Fig. 3.3.21 Plasma Salicylate Profiles For SSA:PVP 1:1 Coprecipitate And Admixture

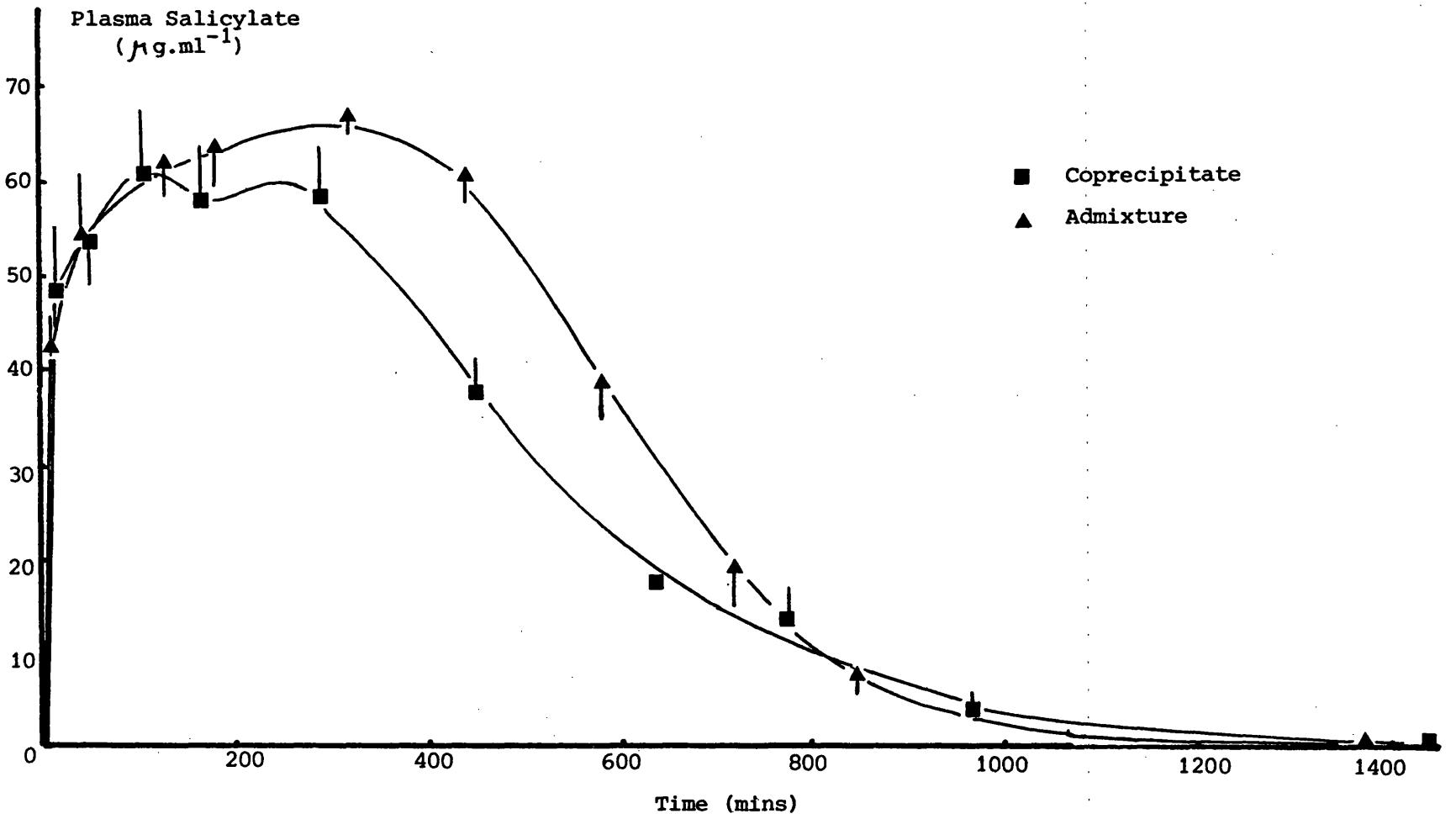


Fig. 3.3.22 Plasma Salicylate Profiles For SSA:PVP 1:2 Coprecipitate And Admixture

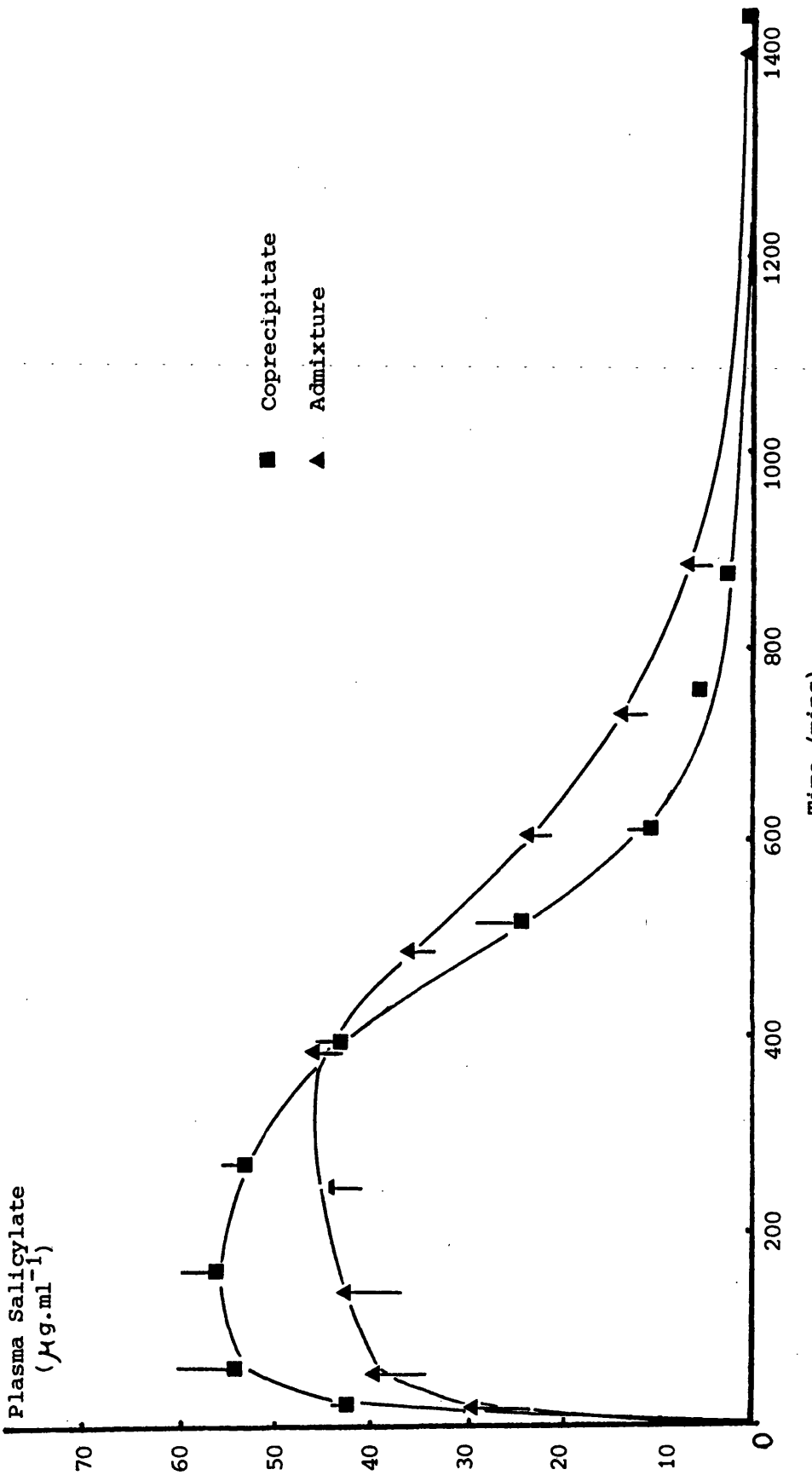


Fig. 3.3.23 Plasma Salicylate Profiles For SSA:PVP 1:5 Coprecipitate And Admixture

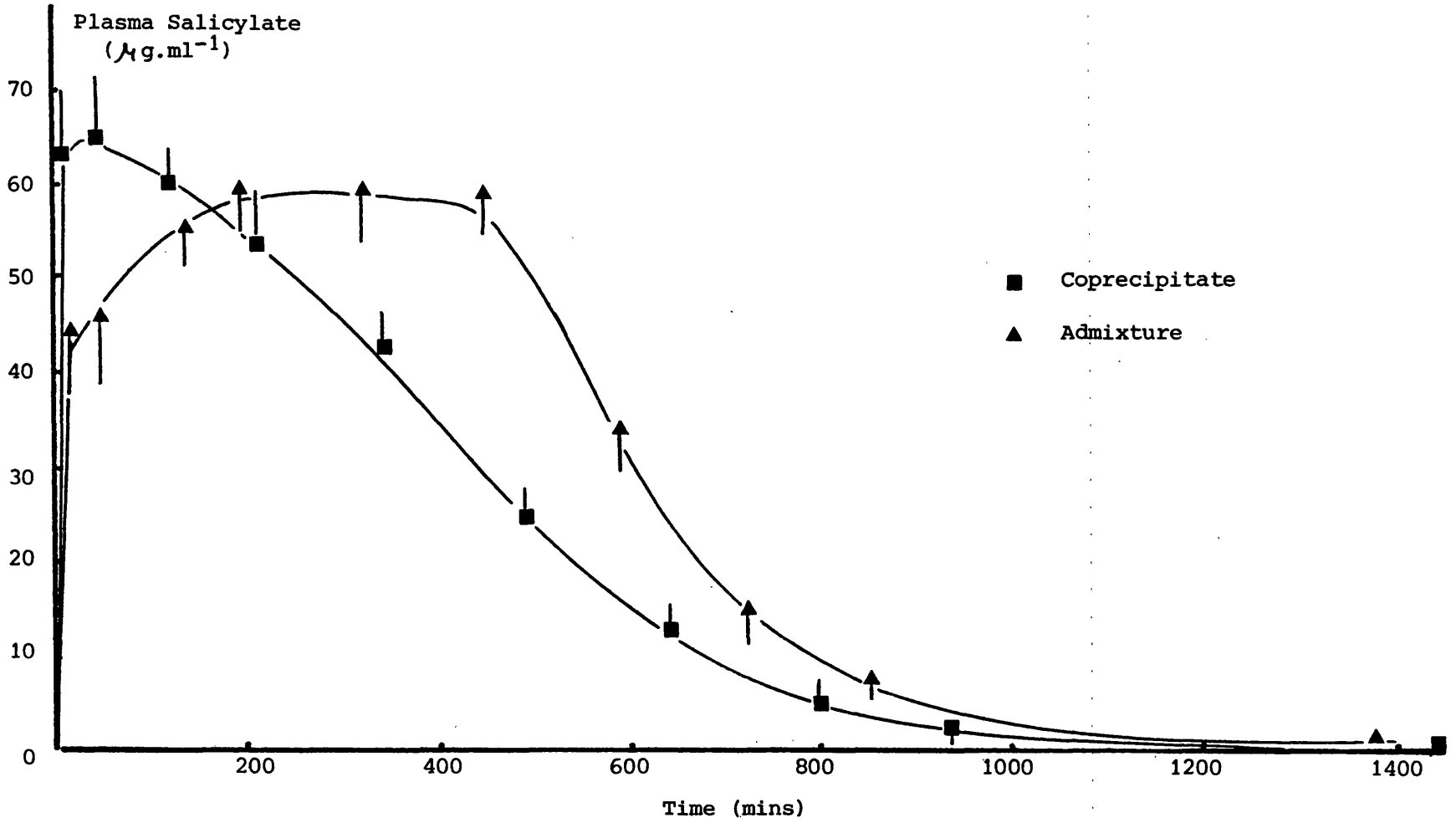


Fig. 3.3.24 Plasma Salicylate Profiles For SSA:PVP 1:10 Coprecipitate And Admixture.

salicylate concentrations and times were interpolated from each profile and are given in Table 3.3.23.

SSA:PVP Ratio	COPRECIPITATE		ADMIXTURE	
	Peak Time (mins)	Peak Conc ($\mu g.ml^{-1}$)	Peak Time (mins)	Peak Conc ($\mu g.ml^{-1}$)
0	100 - 400	53 - 56	100 - 400	53 - 56
2:1	140	69	210	54
1:1	400	58	60	63
1:2	100 (290)	60 (58)	320	67
1:5	170	56	360	46
1:10	45	66	300	60

Table 3.3.23 Approximate Peak Salicylate Times and Concentrations Following Oral Administration of SSA:PVP Coprecipitates and Admixtures and SSA Alone (numbers in parenthesis refer to a second peak).

The area under the plasma level profiles (AUC) for the individual animals receiving each SSA:PVP system (and SSA alone) are given in Tables 3.3.24 and 3.3.25 for the coprecipitates and admixtures respectively.

Variance ratio analysis for the influence of the SSA:PVP systems on the AUC for the animals used is given in Table 3.3.26. For both the SSA:PVP coprecipitates and admixtures the variance due to formulation is not significantly greater than that of biological origin.

Area Under Curve For SSA:PVP Coprecipitates

($\mu g.ml^{-1} \times min$)

Animal \ Ratio	SSA	2:1	1:1	1:2	1:5	1:10
1	50667	33595	28597	36693	-	36150
2	36003	37420	42259	-	-	21800
3	37880	43230	48010	26865	25959	22322
4	41036	40766	41254	29490	31646	36346
5	41220	44239	52918	44589	-	32355
6	36389	34472	39794	31747	28472	27460
Mean	40532	38954	42139	33886	28692	29406
± SE	2222	1832	7531	3127	1646	2672

Table 3.3.24 Area Under Plasma Level Curve Following Oral Administration of SSA and SSA:PVP Coprecipitates

Area Under Curve For SSA:PVP Admixtures

($\mu g.ml^{-1} \times min$)

Animal \ Ratio	SSA	2:1	1:1	1:2	1:5	1:10
1	50667	35650	33519	44004	25031	45384
2	36003	30258	35331	39752	34477	31374
3	37880	28765	51160	38925	31459	32157
4	41036	33318	47124	38768	30224	31666
5	41220	39876	46271	44286	31815	43348
6	36389	-	29996	-	25560	46980
Mean	40532	33173	40567	41147	29761	38485
± SE	2222	1912	3543	1236	1523	3058

Table 3.3.25 Area Under Plasma Level Curve Following Oral Administration of SSA and SSA:PVP Admixtures

	Source of variation	Sum Sq	Degrees of freedom	Mean Sq	F value	F ratio	F Tabulated
SSA:PVP COP	AUC	7.419	4	1.855	5.456	2.36	F_5^4 = 5.19 p'=0.05
	Animal	3.932	5	0.786	2.312		
	Residual	5.438	16	0.340			
	Total	16.788	25				
SSA:PVP ADM	AUC	5.695	4	1.424	3.709	4.63	F_5^4 = 5.19 p'=0,05
	Animal	1.539	5	0.308	0.800		
	Residual	6.920	18	0.385			
	Total	14.15	27				

Table 3.3.26 Variance Ratio Test For SSA:PVP Coprecipitates And Admixtures Following Their Oral Administration - AUC.

The possible influence of PVP on the overall elimination rate of salicylate was studied by examination of the apparent first order elimination rate constants (k_{e1}) and half-lives ($t_{\frac{1}{2}}$), following subjective scrutiny of the first order plots for individual animals. Data points describing the linear elimination phase were submitted to linear least-squares regression analysis and the resultant k_{e1} and $t_{\frac{1}{2}}$ values are given in Tables 3.3.27 - 28 and 3.3.29 - 30 for the coprecipitate and admixture systems respectively. First order plots for the mean (± SE) data of both SSA:PVP systems are shown in Figs. 3.3.31 and 3.3.32.

A variance ratio test (Table 3.3.31) was performed on the elimination rate constants given in Tables 3.3.27 - 30 and shows that for both the coprecipitated and admixed systems the variance associated with the experimental formulation was not significantly greater than that of biological origin.

These results for SSA:PVP systems will be discussed later.

3.3.17 ABSORPTION OF SALICYLATE FROM SSA:CHOLIC ACID (CA) COPRECIPITATES AND ADMIXTURES

The experimental protocol was similar to that for the SSA:PVP systems. Dosage of the SSA:CA coprecipitates was corrected for SSA content according to the % purity data given in Table 2.13.5 and the admixtures were corrected according to their SSA:CA molar ratio. Following oral administration of the SSA:CA 1:10 ratio systems the animals suffered severe gastrointestinal disturbance; therefore, experiments with this ratio were abandoned and the

SSA:PVP Ratio		1	2	3	4	5	6	mean ± SE
2:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	6.462 ±0.55	1.162 * ±0.44	7.964 ±0.62	5.821 ±0.56	3.445 ±0.13	7.304 ±0.57	5.360 ±1.053
	$t_{½}$ (mins)	107	596	87	119	201	95	122±21
	r	0.986	0.876	0.994	0.991	0.996	0.994	-
1:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.907 ±0.14	5.883 ±0.47	3.804 ±0.22	5.035 ±0.29	ND	6.724 ±1.09	5.471 ±0.495
	$t_{½}$ (mins)	117	118	182	138		103	132±14
	r	0.999	0.994	0.997	0.998		0.975	-
1:2	k_{el} ± SE ($x10^{-3}min^{-1}$)	3.536 ±0.16	5.325 ±0.51	4.145 ±0.34		ND	5.543 ±0.45	4.637 ±0.479
	$t_{½}$ (mins)	196	130	167			125	155±16
	r	0.996	0.995	0.997			0.990	

Table 3.3.27 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA:PVP Coprecipitates in Suspension with 4% PVA.
ND = Not Determinable * excluded from mean value.

SSA:PVP Ratio		1	2	3	4	5	6	mean ± SE
1:5	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.677 ±0.36	5.914 ±0.12	5.268 ±0.29				5.620 ±0.189
	$t_{½}$ (mins)	122	117	132				124±4
	r	0.992	0.999	0.995				-
1:10	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.819 ±0.27	8.010 ±0.40	6.361 ±0.74	3.982 ±0.23	3.837 ±0.16	4.498 ±0.29	5.418 ±0.663
	$t_{½}$ (mins)	119	87	109	174	181	154	137±16
	r	0.997	0.998	0.993	0.998	0.996	0.991	-
SSA	k_{el} ± SE ($x10^{-3}min^{-1}$)	4.349 ±0.41	5.747 ±0.82	6.767 ±0.53	4.678 ±0.44	4.405 ±0.91	7.174 ±0.85	5.520 ±0.505
	$t_{½}$ (mins)	159	121	102	148	157	97	131±11
	r	0.996	0.971	0.997	0.996	0.979	0.993	-

Table 3.3.28 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA and SSA:PVP Coprecipitates in Suspension with 4% PVA.

SSA:PVP Ratio		1	2	3	4	5	6	mean ± SE
2:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	3.081 ±0.36	4.370 ±0.15	4.684 ±0.79	5.624 ±0.93	4.914 ±0.05		4.535 ±0.418
	$t_{½}$ (mins)	225	159	148	123	141		159±17
	r	0.993	0.998	0.960	0.974	0.999		
1:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	4.578 ±0.84	5.951 ±0.41	7.032 ±0.16	3.809 ±0.33	3.840 ±0.28	7.287 ±0.49	5.416 ±0.637
	$t_{½}$ (mins)	151	116	99	182	181	95	137±16
	r	0.968	0.998	0.999	0.992	0.995	0.998	
1:2	k_{el} ± SE ($x10^{-3}min^{-1}$)	6.499 ±0.19	5.999 ±0.39	6.748 ±0.96	4.949 ±0.48	4.899 ±0.46		5.819 ±0.385
	$t_{½}$ (mins)	107	116	103	140	142		122±8
	r	0.999	0.996	0.980	0.991	0.996		

Table 3.3.29 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA:PVP Admixtures In Suspension with 4% PVA.

SSA:PVP Ratio		1	2	3	4	5	6	mean ± SE
1:5	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.079 ±0.43	4.875 ±0.18	5.464 ±0.43	ND	5.569 ±0.66	7.204 ±1.42	5.638 ±0.412
	$t_{½}$ (mins)	136	142	127		124	96	125±8
	r	0.993	0.999	0.994		0.986	0.981	-
1:10	k_{el} ± SE ($x10^{-3}min^{-1}$)	3.337 ±0.13	6.184 ±0.62	7.528 ±0.78	6.108 ±0.31	5.228 ±0.19	4.539 ±0.49	5.487 ±0.595
	$t_{½}$ (mins)	208	112	92	114	133	153	135±17
	r	0.998	0.990	0.989	0.998	0.998	0.989	-

Table 3.3.30 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA:PVP Admixtures in Suspension with 4% PVA. ND = Not Determinable

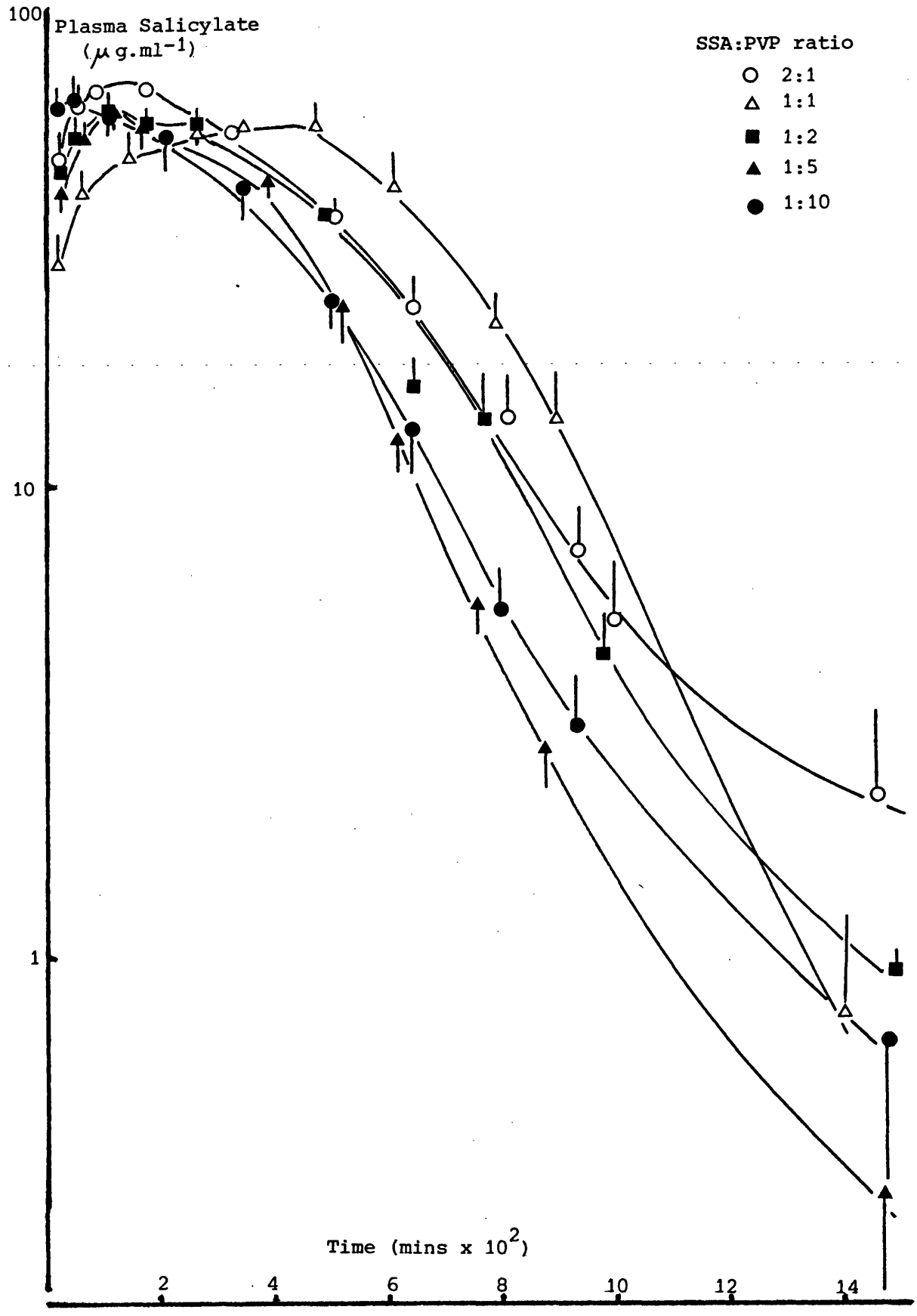


Fig. 3.3.31 Plasma Salicylate (log scale) vs Time Profiles For SSA:PVP Coprecipitates

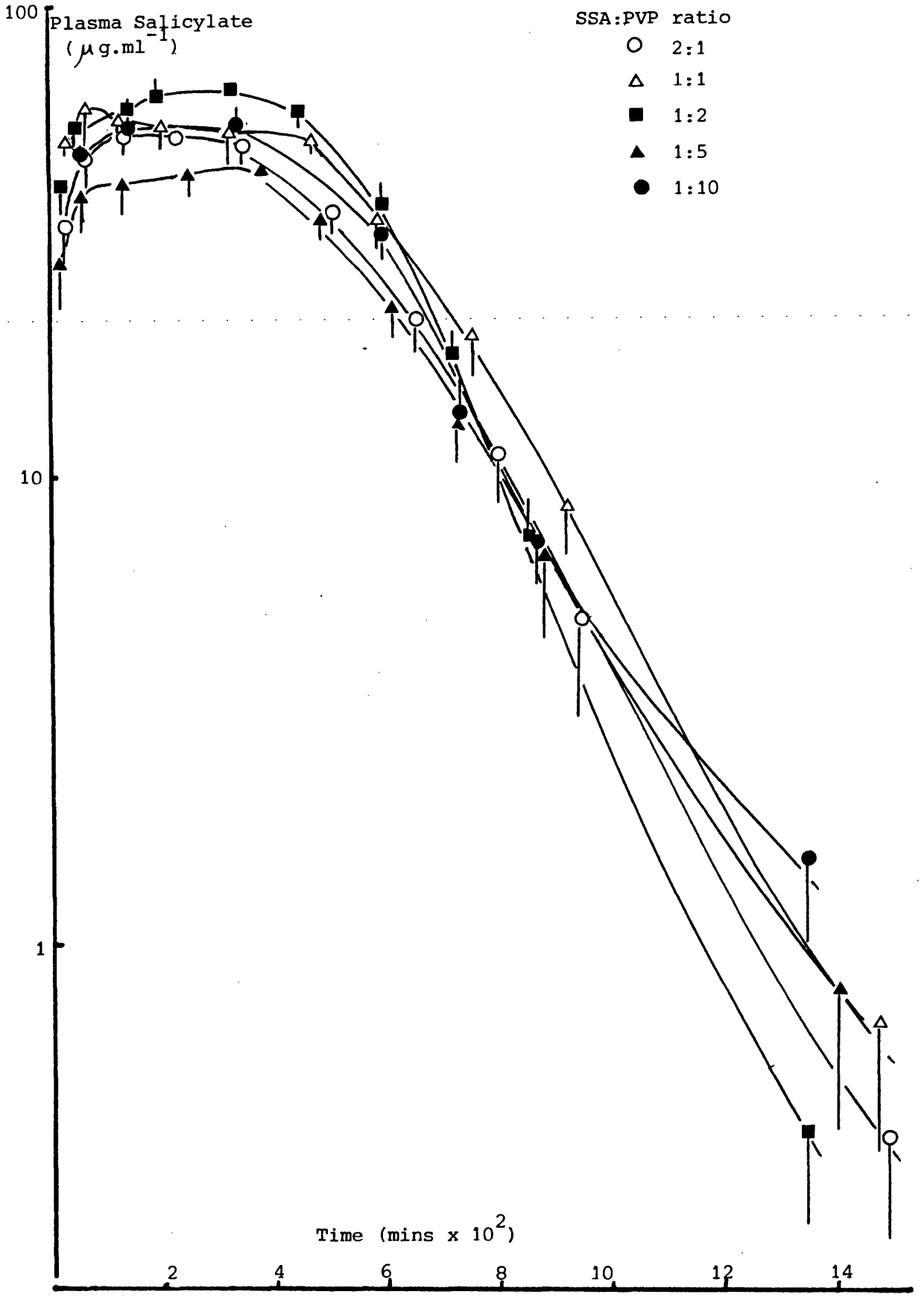


Fig. 3.3.32 Plasma Salicylate (log scale) vs Time Profiles For SSA:PVP Admixtures.

	Source of Variation	Sum Sq.	Degrees of Freedom	Mean Sq.	F Value	F Ratio	F Tabulated
SSA:PVP COP	k_{el}	6.911	4	1.728	0.554	2.65	$F_4^5 = 6.26$ $p' = 0.05$
	Animal	22.91	5	4.589	1.469		
	Residual	43.68	14	3.119			
	Total	73.49	23				
SSA:PVP ADM	k_{el}	5.187	4	1.297	1.046	2.06	$F_4^5 = 6.26$ $p' = 0.05$
	Animal	13.36	5	2.672	2.155		
	Residual	21.07	17	1.240			
	Total	39.67	26				

Table 3.3.31 Variance Ratio Data For SSA:PVP Coprecipitates and Admixtures - Elimination

animals sacrificed. The significance of the effect of non-physiological concentrations of bile acids on the gastrointestinal tract will be discussed later.

The mean ($\pm$ SE) plasma salicylate concentration vs time profiles for the 2:1, 1:1, 1:2 and 1:5 ratio coprecipitated and admixed systems are shown in Figs. 3.3.33 to 3.3.36.

Peak salicylate concentrations and times, obtained by interpolation from the appropriate Figures are given in Table 3.3.32.

SSA:CA Ratio	COPRECIPITATE		ADMIXTURE	
	Peak Time (mins)	Peak Conc. ($\mu g.ml^{-1}$)	Peak Time (mins)	Peak Conc. ($\mu g.ml^{-1}$)
0	100 - 400	53 - 56	100 - 400	53 - 56
2:1	180	62	150 - 550	49 - 52
1:1	45	84	45	88
1:2	60 (330)	60 (58)	380	57
1:5	350	58	350	67

Table 3.3.32 Peak Salicylate Times and Concentrations for SSA and SSA:CA Coprecipitates and Admixtures Following Their Oral Administration to the Rat. (Numbers in parenthesis refer to a second peak).

The area under the plasma level curves (AUC) for the individual animals receiving each SSA:CA system (and SSA alone) are given in Tables 3.3.33 and 3.3.34 for the coprecipitates and admixtures respectively.

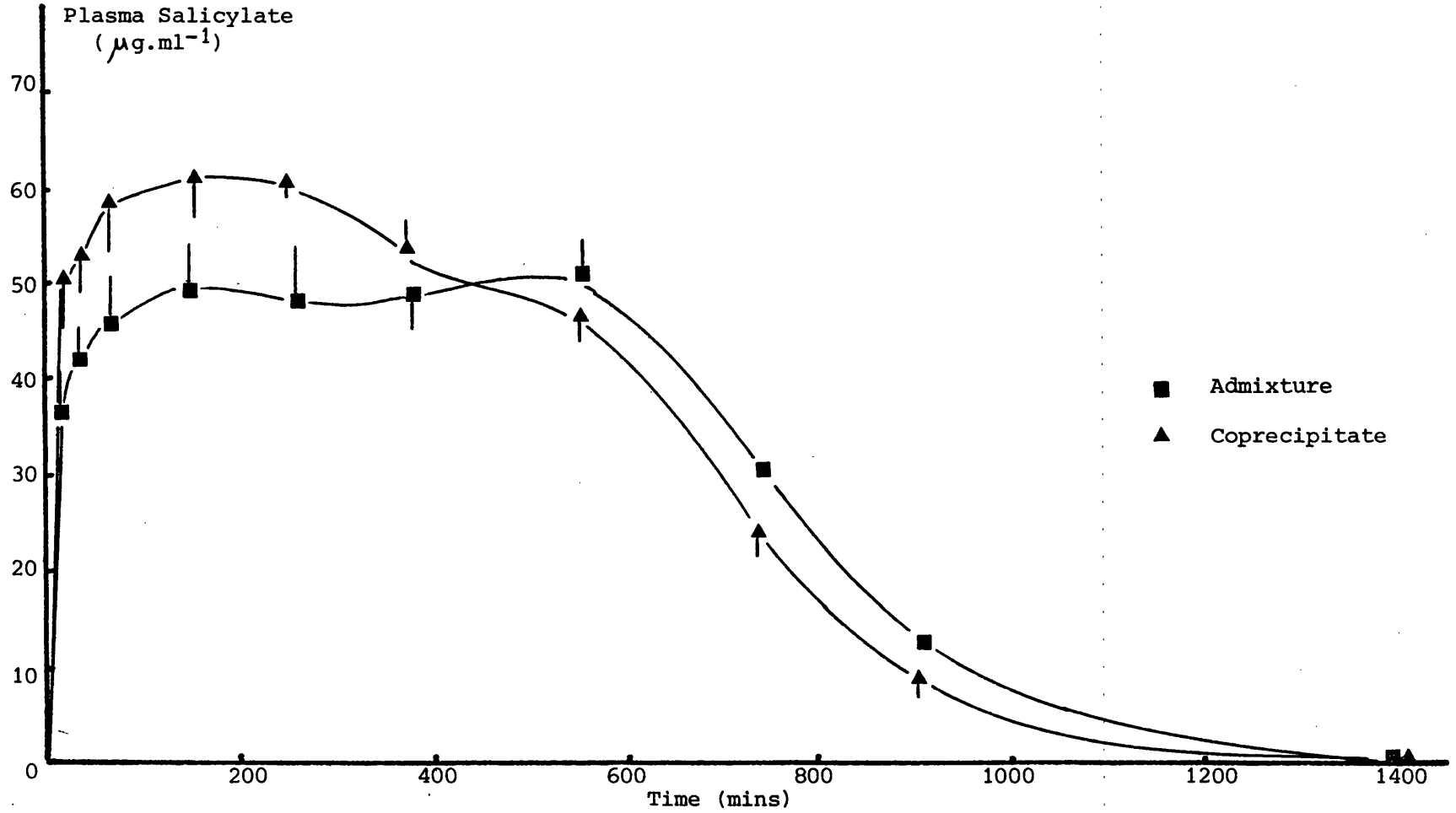


Fig. 3.3.33 Plasma Salicylate Profiles For SSA:CA 2:1 Coprecipitate And Admixture

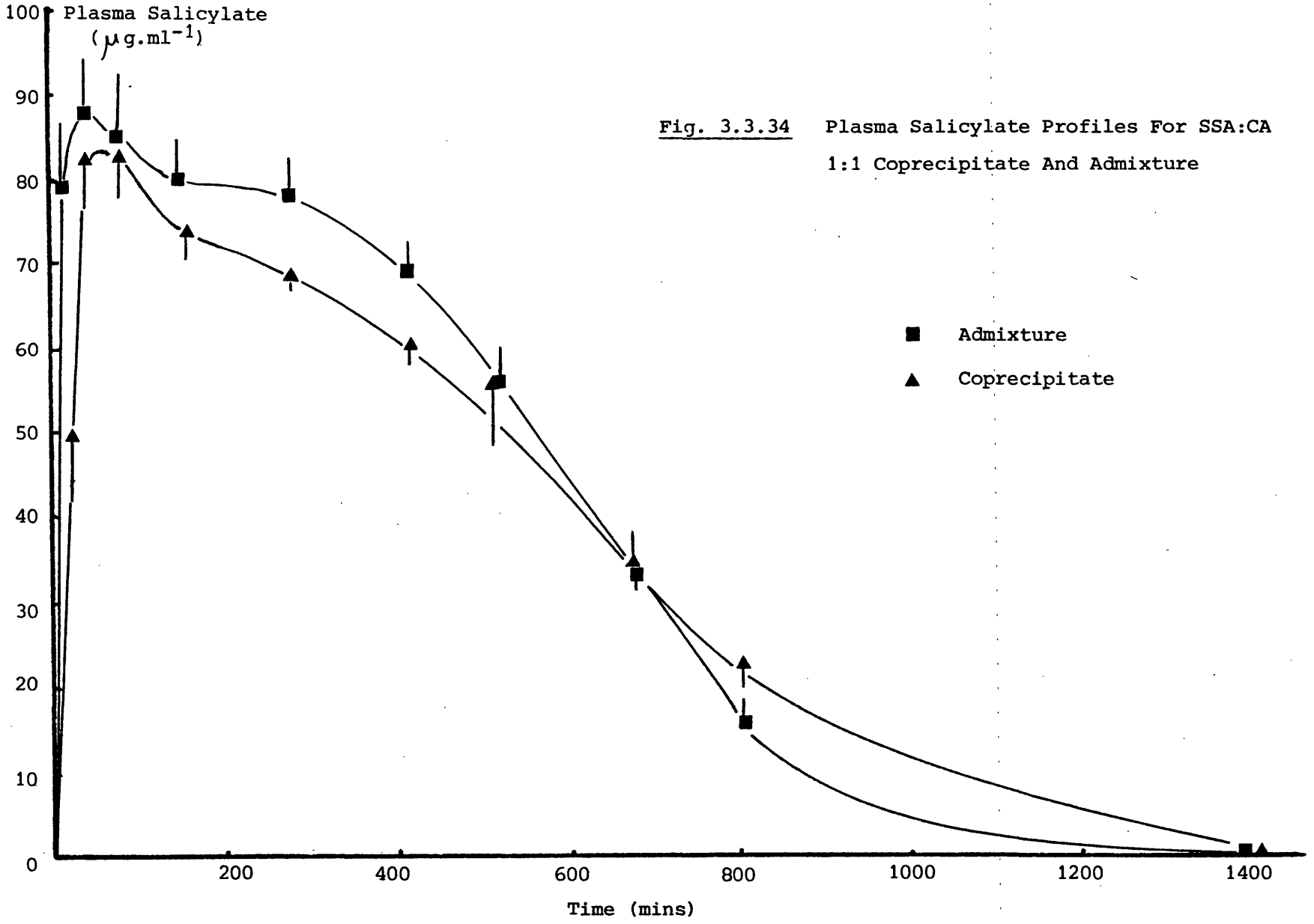


Fig. 3.3.34 Plasma Salicylate Profiles For SSA:CA 1:1 Coprecipitate And Admixture

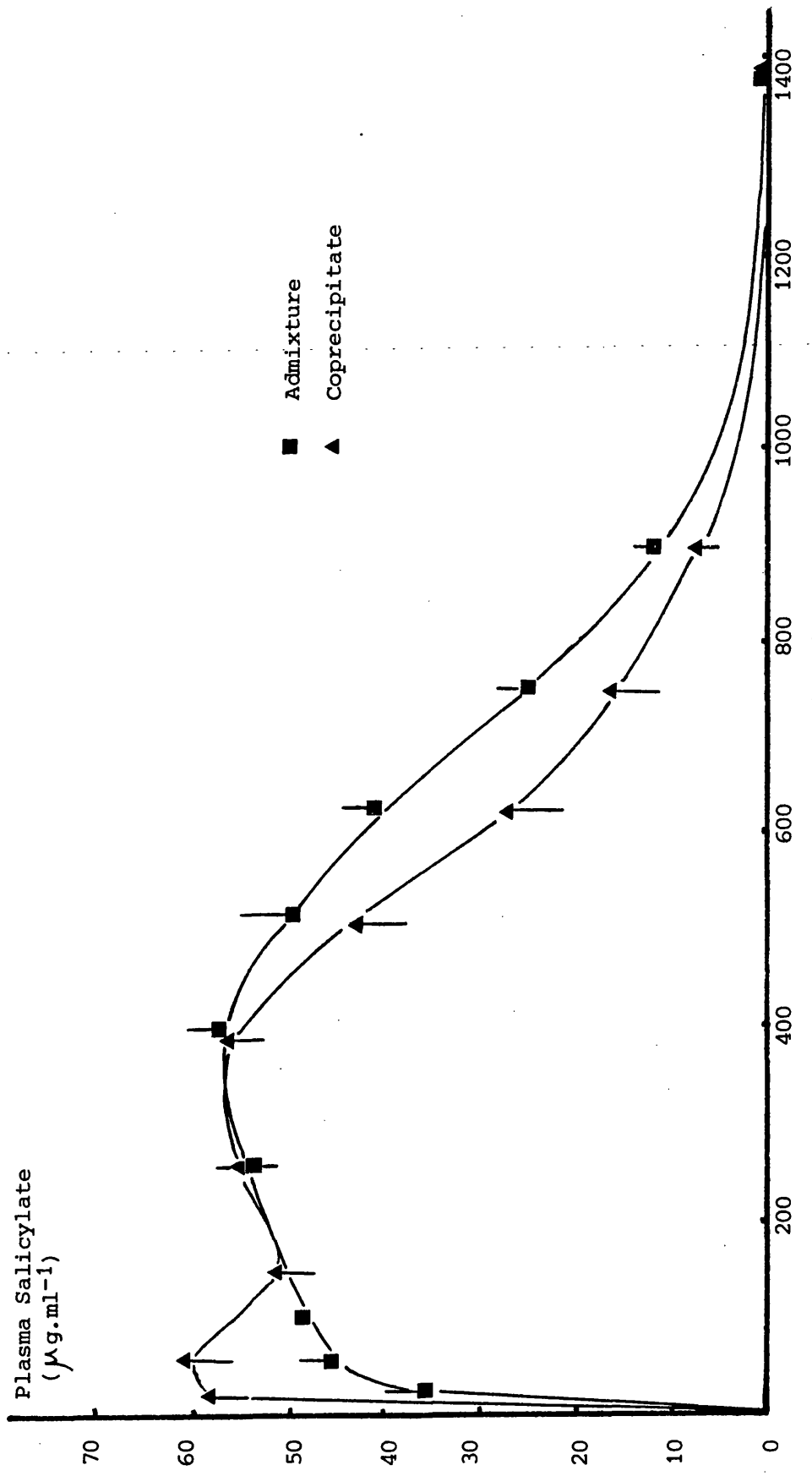


Fig. 3.3.35 Plasma Salicylate Profiles For SSA:CA 1:2 Coprecipitate And Admixture.

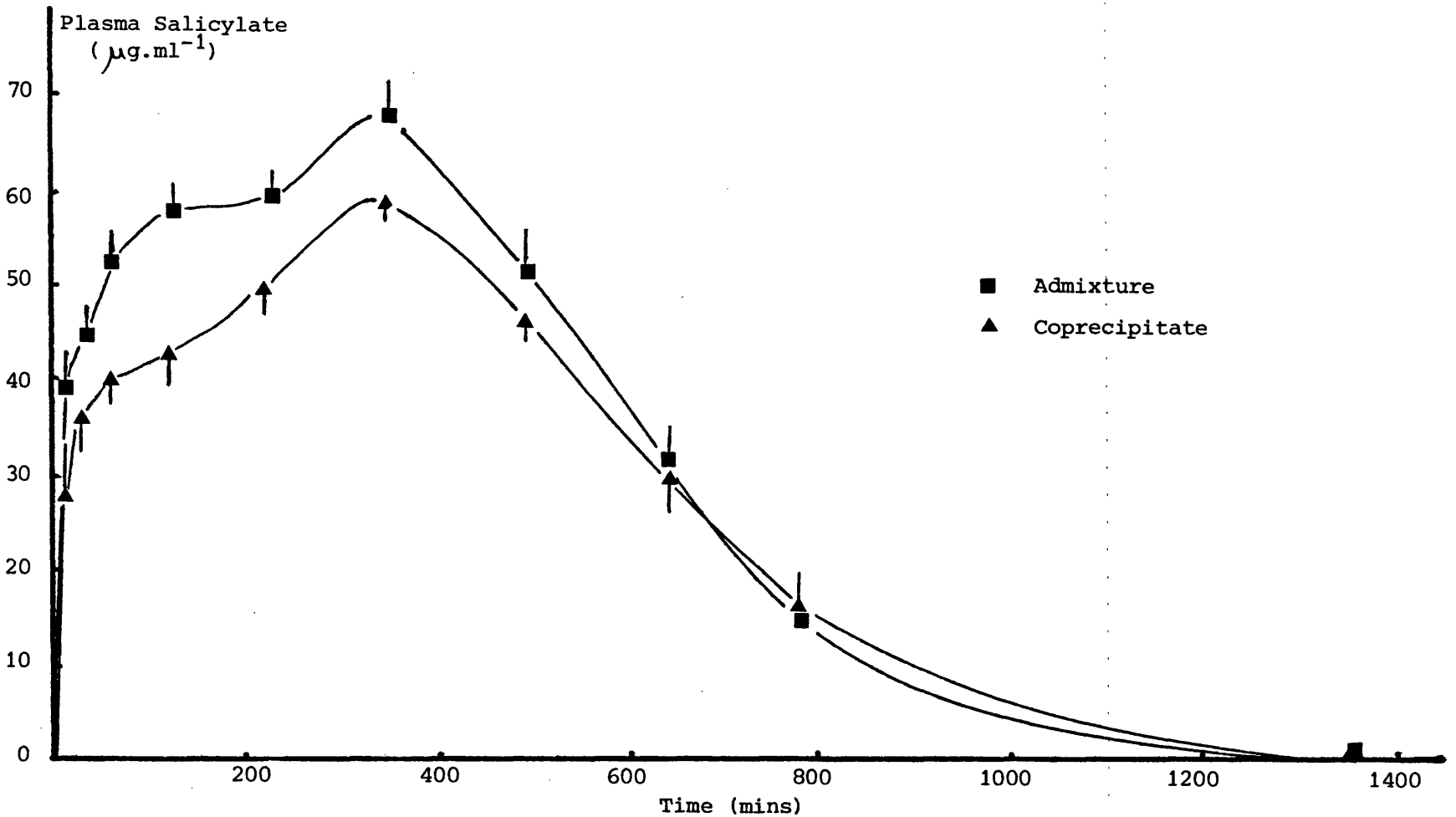


Fig. 3.3.36 Plasma Salicylate Profiles For SSA:CA 1:5 Coprecipitate And Admixture

Area Under Curve For SSA:CA Coprecipitates

($\mu g.ml^{-1} \times min$)

Animal \ Ratio	SSA	2:1	1:1	1:2	1:5
1	50667	36941	49980	51518	30592
2	36003	41562	54259	37099	34999
3	37880	37526	47258	35548	36948
4	41036	45036	49633	32752	-
5	41220	46504	58552	31331	42486
6	36389	45231	56490	35866	38849
Mean	40532	42133	52695	37352	36775
± SE	2222	1690	1803	2964	1979

Table 3.3.33 Area Under Plasma Level Curve Following Oral Administration of SSA and SSA:CA Coprecipitates

Area Under Curve for SSA:CA Admixtures

($\mu g.ml^{-1} \times min$)

Animal \ Ratio	SSA	2:1	1:1	1:2	1:5
1	50667	46443	52903	36017	36058
2	36003	31527	49935	40881	43715
3	37880	47235	54916	32987	47837
4	41036	39534	-	42153	36265
5	41220	45679	51173	46120	43375
6	36389	40217	61507	44207	41457
Mean	40532	41773	54087	40394	41452
± SE	2222	2445	2036	2039	1876

Table 3.3.34 Area Under Plasma Level Curve Following Oral Administration of SSA and SSA:CA Admixtures

Variance ratio analysis for the influence of the SSA:CA systems on the AUC for the animals used is given in Table 3.3.35. For both the SSA:CA coprecipitated and admixed systems the variation in AUC associated with the formulation is significantly greater than that of animal origin, indicating that the coprecipitate and admixture produced systems changes in bioavailability as determined by AUC analysis.

A Student 't' test comparison between the AUC data for the SSA:CA coprecipitates and admixtures is given in Table 3.3.36 and shows that the 1:1 ratio coprecipitate and admixture has resulted in a significantly enhanced AUC in comparison to SSA alone and to all other SSA:CA systems. All systems apart from the 1:1 ratio showed no statistically distinguishable differences. These results will be discussed later and possible explanations offered.

The influence of cholic acid on the overall elimination rate constant for salicylate was studied by examination of the rate constants (k_{e1}) and corresponding half-lives ($t_{\frac{1}{2}}$), following subjective scrutiny of the first order plots for individual animals. Data points describing the linear elimination phase were submitted to least-squares regression analysis; the resultant k_{e1} and $t_{\frac{1}{2}}$ values are given in Tables 3.3.37 - 39 for both the coprecipitates and admixtures. First order plots of the mean ($\pm$ SE) plasma salicylate levels for both SSA:CA systems are shown in Figs. 3.3.37 and 3.3.38.

Variance ratio analysis performed on the elimination rate

	Source of Variation	Sum Sq.	Degrees of Freedom	Mean Sq.	F Value	F Ratio	F Tabulated
COPRECIPITATES	AUC	7.097	3	2.366	8.542	10.91	$F_5^3 = 5.41$ $p' = 0.05$
	Animal	1.084	5	0.217	0.783		
	Residual	3.384	14	0.277			
	Total	12.06	22				
ADMIXTURES	AUC	9.998	3	3.333	10.514	19.84	$F_5^3 = 5.41$ $p' = 0.05$
	Animal	0.838	5	0.168	0.530		
	Residual	4.442	14	0.317			
	Total	15.29	22				

Table 3.3.35 Variance Ratio Data For SSA:CA Coprecipitates and Admixtures - AUC

	Ratio	0	2:1	1:1	1:2
COPRECIPITATE	2:1	0.574 N = 12			
	1:1	4.250 N = 12	4.275 N = 12		
	1:2	0.858 N = 12	1.401 N = 12	4.423 N = 12	
	1:5	1.263 N = 11	2.060 N = 11	5.948 N = 11	0.162 N = 11
ADMIXTURE	2:1	0.375 N = 12			
	1:1	4.498 N = 11	3.871 N = 11		
	1:2	0.046 N = 12	0.433 N = 12	4.753 N = 11	
	1:5	0.316 N = 12	0.104 N = 12	4.564 N = 11	0.382 N = 12

For N = 11 t_{tab} = 2.262 p' = 0.05

N = 12 t_{tab} = 2.228 p' = 0.05

Table 3.3.36 Student 't' Test Comparison Between AUC Data For SSA:CA Coprecipitates and Admixtures with SSA (75 - 105μm) Alone.

SSA:CA Ratio		1	2	3	4	5	6	mean ± SE
2:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.821 ±0.26	5.790 ±1.28	5.971 ±0.78	5.417 ±0.52	5.163 ±0.24	7.250 ±0.54	5.902 ±0.296
	$t_{½}$ (mins)	119	120	116	128	134	96	119±5
	r	0.998	0.977	0.992	0.995	0.999	0.997	-
1:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	ND	ND	ND	4.127 ±0.340	4.147 ±0.27	5.110 ±0.38	4.461 ±0.325
	$t_{½}$ (mins)				168	167	136	157±11
	r				0.997	0.998	0.995	-
1:2	k_{el} ± SE ($x10^{-3}min^{-1}$)	ND	5.699 ±0.03	5.303 ±0.14	6.987 ±0.34	4.913 ±0.15	5.658 ±0.31	5.712 ±0.349
	$t_{½}$ (mins)		122	131	99	141	123	123±7
	r		0.999	0.999	0.997	0.998	0.997	-

Table 3.3.37 Apparent First Order Elimination Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA:CA Coprecipitates In Suspension with 4% PVA. (ND Not Determinable)

SSA:CA Ratio		1	2	3	4	5	6	mean ± SE
2:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	ND	8.050 ±0.36	ND	3.958 ±0.47	4.389 ±0.60	3.643 ±0.19	5.010 ±1.025
	$t_{½}$ (mins)		86		175	158	190	152±23
	r		0.999		0.993	0.991	0.999	-
1:1	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.518 ±0.61	5.204 ±0.29	ND	-	ND	5.171 ±0.20	5.298 ±0.111
	$t_{½}$ (mins)	126	133		-		134	131±3
	r	0.994	0.997		-		0.999	-
1:2	k_{el} ± SE ($x10^{-3}min^{-1}$)	6.763 ±1.11	7.385 ±0.38	7.380 ±0.39	4.915 ±0.28	4.164 ±0.51	4.336 ±0.27	5.824 ±0.620
	$t_{½}$ (mins)	103	94	94	141	166	160	126±14
	r	0.987	0.999	0.997	0.998	0.993	0.998	-

Table 3.3.38 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA:CA Admixtures In Suspension with 4% PVA. (ND = Not Determinable)

SSA:CA Ratio		1	2	3	4	5	6	mean ± SE
1:5 COP	k_{el} ± SE ($x10^{-3}min^{-1}$)	ND	4.260 ±0.56	4.261 ±0.03	-	ND	ND	4.441 ±0.180
	$t_{½}$ (mins)		163	150	-			156±6
	r		0.991	0.999	-			-
1:5 ADM	k_{el} ± SE ($x10^{-3}min^{-1}$)	6.409 ±0.38	4.280 ±0.59	4.803 ±0.76	4.863 ±0.13	4.549 ±0.95	5.462 ±0.97	5.061 ±0.314
	$t_{½}$ (mins)	108	162	144	143	152	126	139±8
	r	0.998	0.991	0.988	0.999	0.979	0.985	-

Table 3.3.39 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA:CA 1:5 Coprecipitate and Admixture In Suspension with 4% PVA. (ND = Not Determinable)

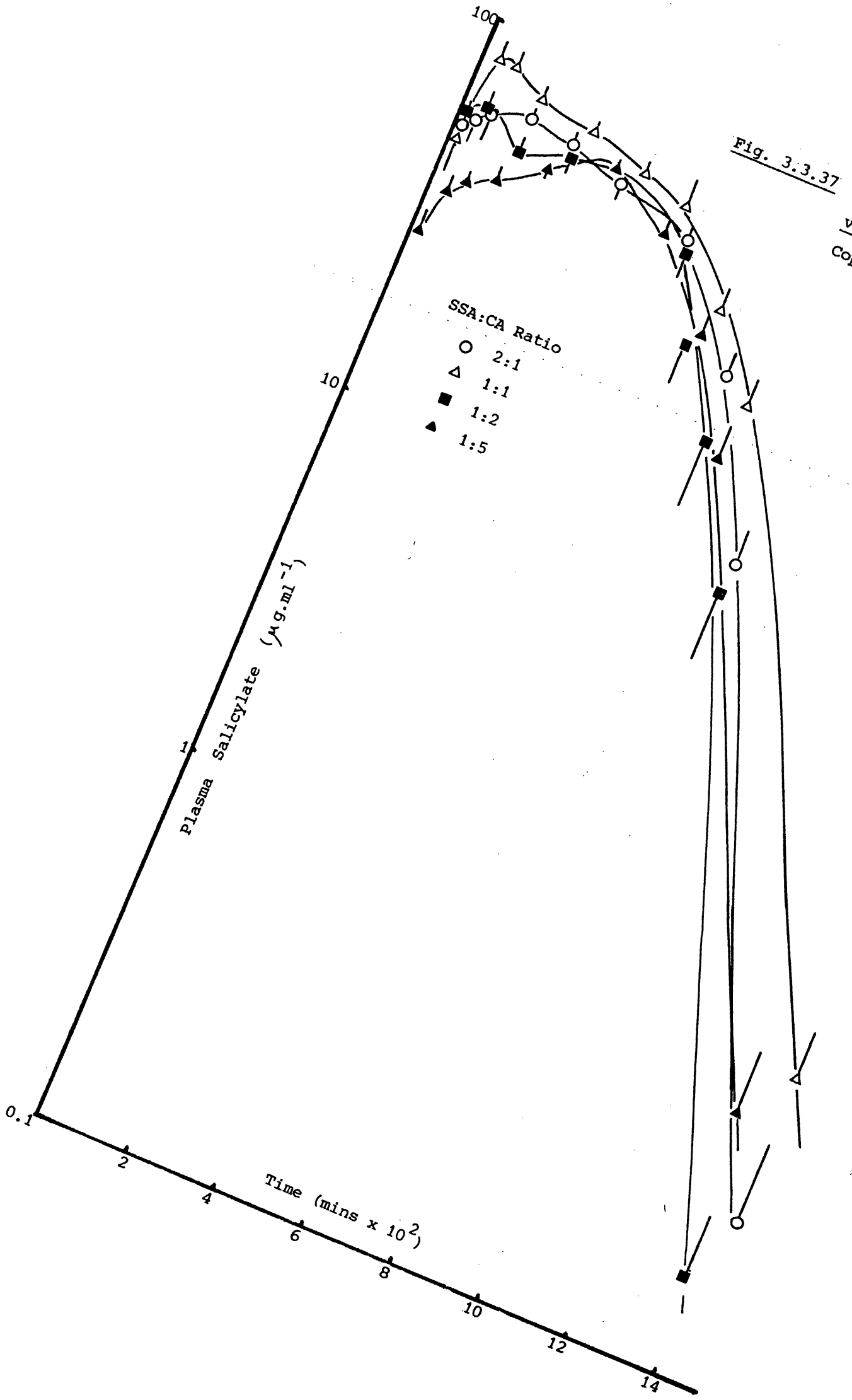


Fig. 3.3.37 Plasma Salicylate (log scale) vs Time Profiles For SSA:CA Coprecipitates.

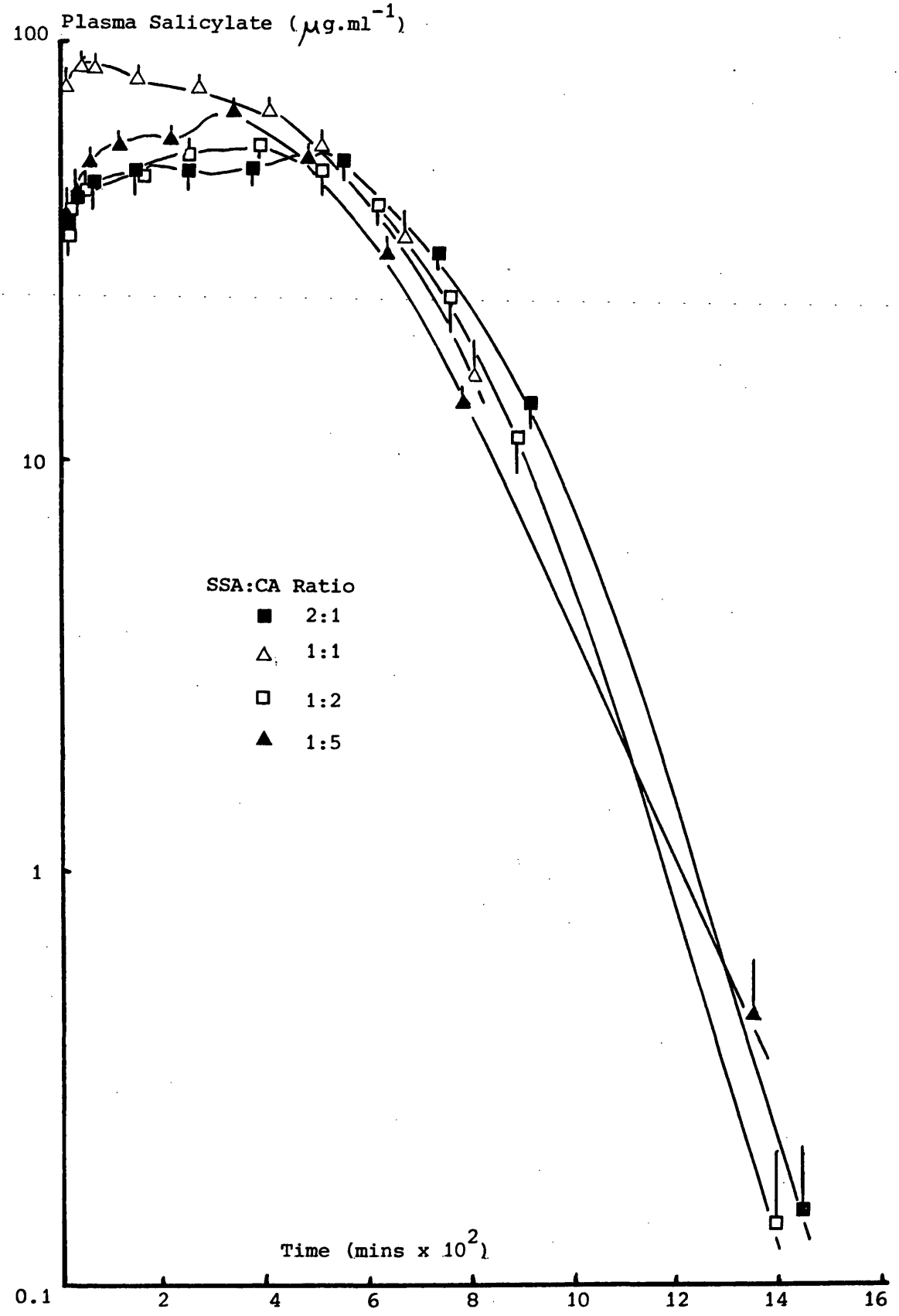


Fig. 3.3.38 Plasma Salicylate (Log Scale) vs Time Profiles For SSA:CA Admixtures.

constants for both SSA:CA systems, the results of which are given in Table 3.3.40, shows SSA coprecipitates and admixtures with cholic acid to have had no significant influence on the elimination of salicylate.

3.3.18 ABSORPTION OF SALICYLATE FROM SSA:DEOXYCHOLIC ACID (DOCA) COPRECIPITATES AND ADMIXTURES

The experimental protocol was similar to that for the SSA:PVP systems. Dosage of the SSA:DOCA coprecipitates was corrected for SSA content according to the % purity data given in Table 2.13.6 and the admixtures were corrected according to their SSA:DOCA molar ratio. Administration of SSA:DOCA systems of a ratio greater than 1:2 were abandoned due to severe gastro-intestinal disturbance of the animals which were subsequently sacrificed.

The mean ($\pm$ SE) plasma salicylate vs time profiles for the SSA:DOCA 2:1 and 1:1 systems are shown in Figs. 3.3.39 and 3.3.40 respectively. The truncated profiles for the 1:2 and 1:5 admixtures are shown in Fig. 3.3.41. These profiles are not complete due to the sacrifice of the animals before completion of the experiment because of gastrointestinal disturbance.

Peak salicylate concentrations and times, interpolated from these profiles are given in Table 3.3.41.

	Source of variation	Sum Sq	Degrees of Freedom	Mean Sq	F value	F ratio	F Tabulated
SSA:CA COP	K_{el}	11.000	3	3.668	8.026	5.26	F_5^3 = 5.41 p'=0.05
	Animal	3.491	5	0.698	1.527		
	Residual	3.201	7	0.457			
	Total	17.70	15				
SSA:CA ADM	K_{el}	3.150	3	1.050	0.755	3.45	F_3^5 = 9.01 p'=0.05
	Animal	18.10	5	3.620	2.602		
	Residual	13.91	10	1.391			
	Total	35.16	18				

Table 3.3.40 Variance Ratio Test For SSA:CA Coprecipitates And Admixtures - Elimination.

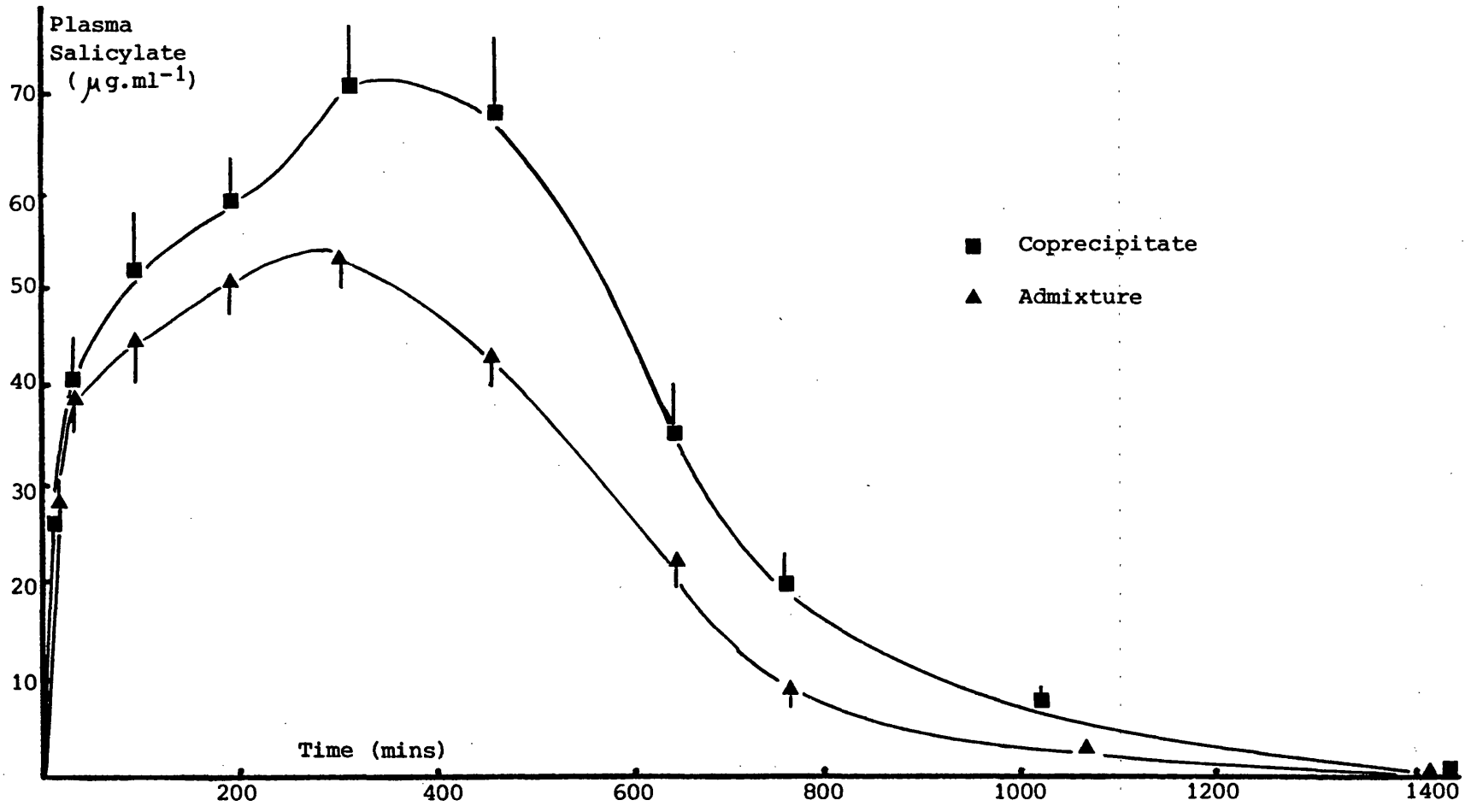


Fig. 3.3.39 Plasma Salicylate Profiles For SSA:DOCA 2:1 Coprecipitate And Admixture

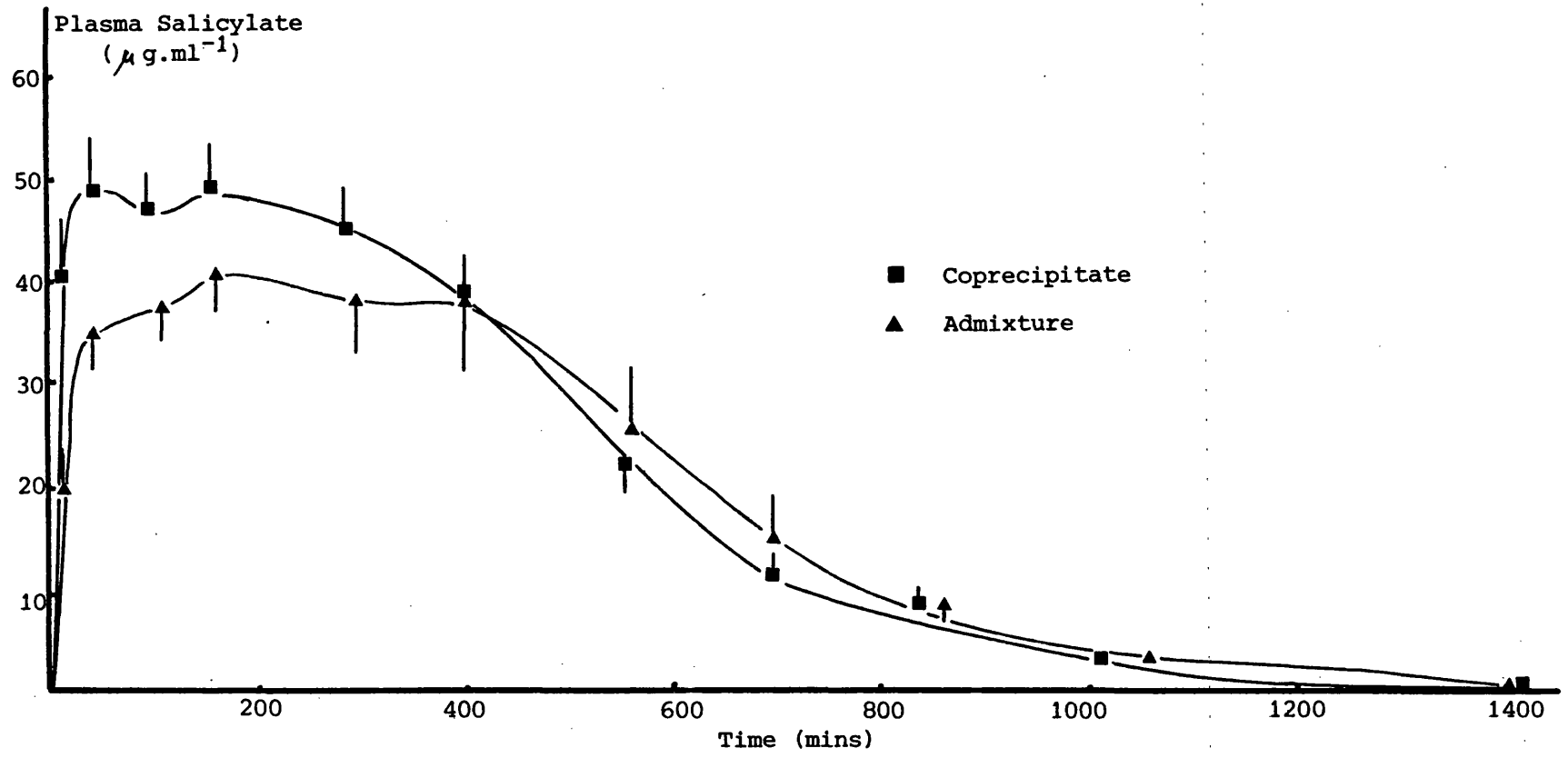


Fig. 3.3.40 Plasma Salicylate Profiles For SSA:DOCA 1:1 Coprecipitate And Admixture.

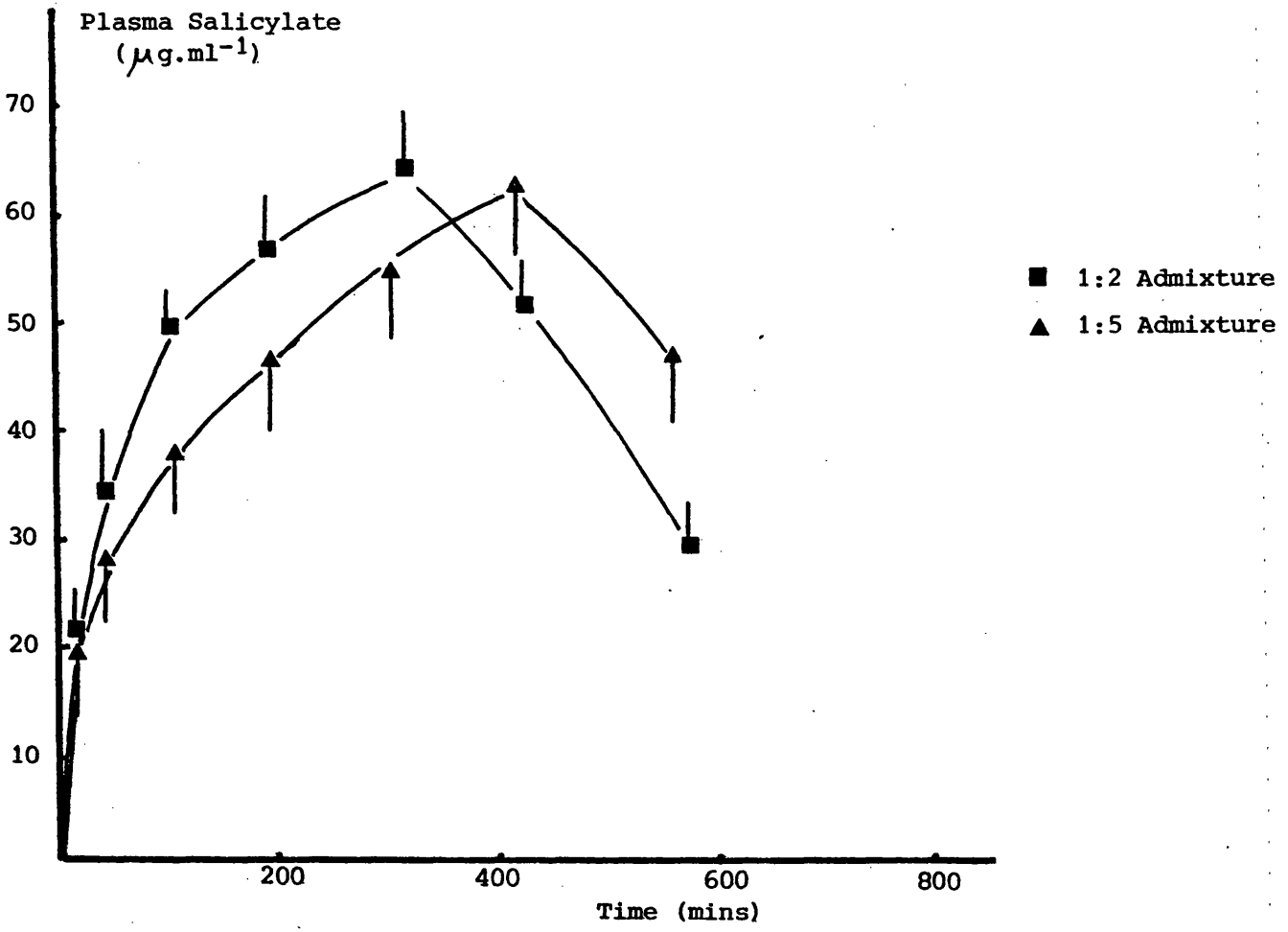


Fig. 3.3.41 Plasma Salicylate Profiles For SSA:DOCA 1:2 And 1:5 Admixtures

SSA:DOCA Ratio	COPRECIPITATE		ADMIXTURE	
	Peak Time (Mins)	Peak Conc. ($\mu g.ml^{-1}$)	Peak Time (Mins)	Peak Conc. ($\mu g.ml^{-1}$)
0	100 - 400	53 - 56	100 - 400	53 - 56
2:1	300	71	300	53
1:1	50 - 150	47 - 49	150 - 400	38 - 41
1:2	-	-	310	64
1:5	-	-	420	62

Table 3.3.41 Peak Salicylate Concentration and Time for SSA:DOCA Coprecipitates and Admixtures, and SSA Alone, Following Their Oral Administration to the Rat.

The area under the plasma level curve (AUC) for the individual animals receiving each SSA:DOCA system and SSA alone are given in Table 3.3.42. A Student 't' test comparison between these AUC data is give in Table 3.3.43.

The Student 't' test analysis shows that apart from the 2:1 coprecipitate the other systems (2:1, 1:1 admixtures and 1:1 coprecipitate) exhibit a significantly reduced AUC in comparison to SSA alone, and, that these systems are not significantly different from each other. This reduced absorption will be discussed later.

The possible influence of deoxycholic acid on the overall elimination rate constant for salicylate was studied by examina-

Area Under Curve For SSA:DOCA Systems

(μ g.ml^{-1} x min)

Animal \ Ratio	SSA	2:1 COP	2:1 ADM	1:1 COP	1:1 ADM
1	50667	31553	26748	29779	-
2	36003	54188	35579	26409	27920
3	37880	54790	33504	20530	40072
4	41036	44848	34686	36158	25773
5	41220	44093	30407	32485	16076
6	36389	45242	29084	23349	19974
Mean	40532	45786	31668	28118	25963
± SE	2222	3456	1417	2379	4102

Table 3.3.42 Area Under Plasma Level Curve Following Oral Administration of SSA and SSA:DOCA, 2:1 and 1:1 Coprecipitates and Admixtures

Ratio	0	2:1 COP	2:1 ADM	1:1 COP
2:1 COP	1.279 N = 12			
2:1 ADM	3.363 N = 12	3.780 N = 12		
1:1 COP	3.814 N = 12	4.211 N = 12	1.282 N = 12	
1:1 ADM	3.122 N = 11	3.696 N = 11	0.315 N = 11	0.455 N = 11

For N = 11 t_{tab} = 2.262 p' = 0.05

N = 12 t_{tab} = 2.228 p' = 0.05

Table 3.3.43 Student 't' Test Comparison Between AUC Data For SSA:DOCA 2:1 and 1:1 Ratio Coprecipitates And Admixtures With SSA (75 - 105μm) Alone.

tion of k_{e1} and half-life data obtained from first order plots for individual animals. Following subjective scrutiny of these first order plots, data points describing the linear elimination phase were submitted to least-squares regression analysis; the resultant k_{e1} and $t_{\frac{1}{2}}$ values are given in Tables 3.3.44 and 3.3.45 for the SSA:DOCA 2:1 and 1:1 systems respectively. First order plots of the mean ($\pm$ SE) plasma salicylate levels for both SSA:DOCA ratios are shown in Figs. 3.3.41 and 3.3.42 respectively. The latter figure also includes the truncated profiles for the 1:2 and 1:5 admixtures. The elimination rate constants are compared to SSA alone and to each other by a Student 't' test the results of which are given in Table 3.3.46.

		1	2	3	4	5	6	mean ± SE
2:1 COP	k_{el} ± SE ($x10^{-3}min^{-1}$)	4.152 ±0.57	3.971 ±0.16	4.804 ±0.72	3.578 ±0.05	3.205 ±0.15	4.138 ±0.31	3.975 ±0.223
	$t_{½}$ (mins)	167	175	144	194	217	168	178±10
	r	0.981	0.998	0.979	0.999	0.997	0.997	-
2:1 ADM	k_{el} ± SE ($x10^{-3}min^{-1}$)	5.690 ±0.60	4.756 ±0.78	3.978 ±0.18	4.393 ±0.67	3.616 ±0.55	5.257 ±0.30	4.615 ±0.318
	$t_{½}$ (mins)	122	146	174	158	192	132	154±11
	r	0.989	±0.974	0.997	0.978	0.977	0.997	-

Table 3.3.44 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficients (r) Following Oral Administration of SSA:DOCA 2:1 Coprecipitate and Admixture In Suspension with 4% PVA.

		1	2	3	4	5	6	mean ± SE
1:1 COP	k_{el} ± SE ($x10^{-3}min^{-1}$)	3.120 ±0.50	2.667 ±0.54	3.596 ±0.41	3.807 ±0.13	3.935 ±0.27	3.945 ±0.19	3.512 ±0.210
	$t_{½}$ (mins)	222	260	193	182	176	176	202±14
	r	0.952	0.975	0.975	0.998	0.991	0.996	-
1:1 ADM	k_{el} ± SE ($x10^{-3}min^{-1}$)	3.840 ±0.42	4.368 ±0.22	5.009 ±0.17	4.356 ±0.15	2.941 ±0.35	- -	4.103 ±0.345
	$t_{½}$ (mins)	181	159	139	159	236	-	175±17
	r	0.983	0.997	0.999	0.998	0.981	-	-

Table 3.3.45 Apparent First Order Elimination Rate Constants (k_{el}), Half Lives ($t_{½}$) and Correlation Coefficient (r) Following Oral Administration of SSA:DOCA 1:1 Coprecipitate and Admixture In Suspension with 4% PVA.

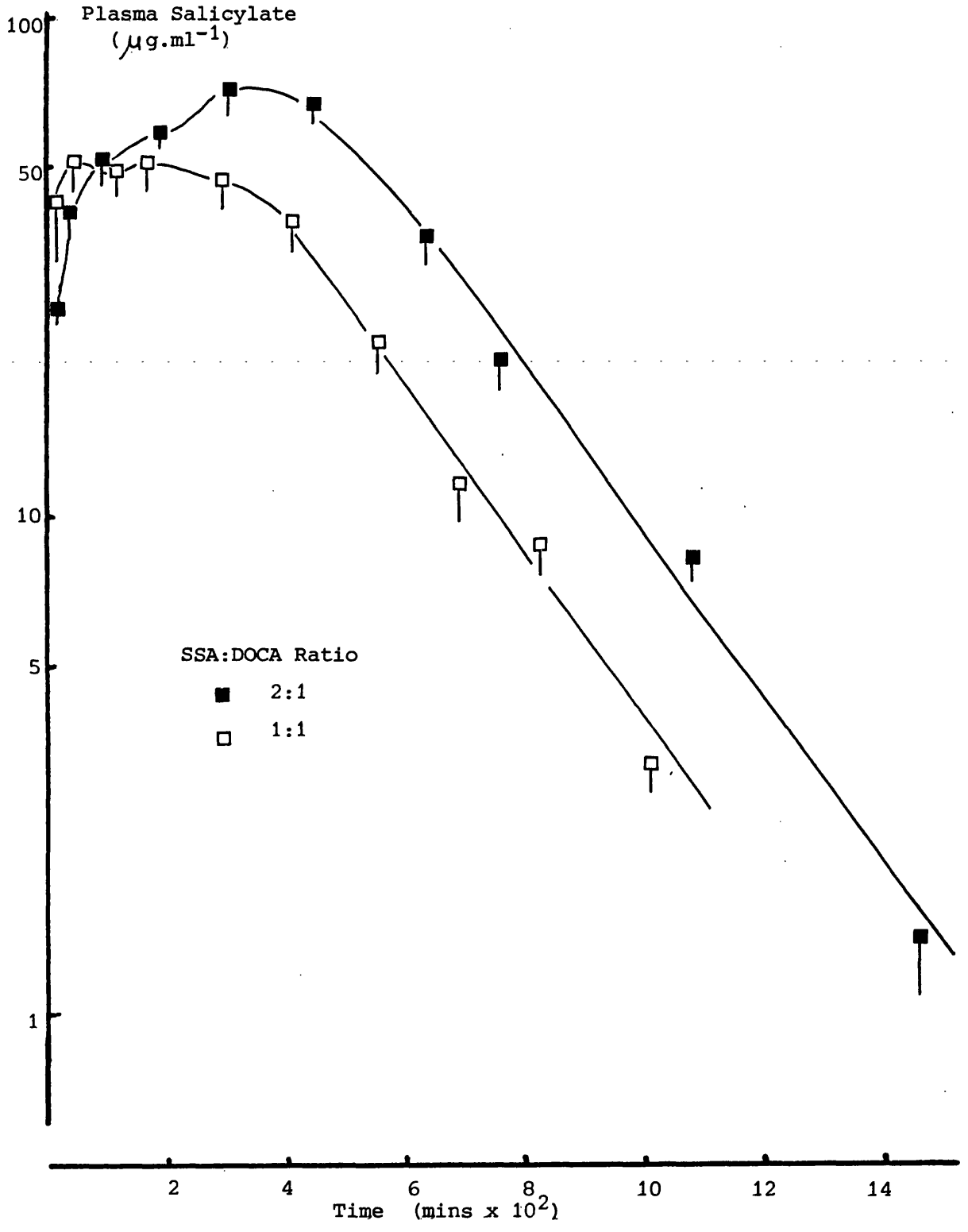


Fig. 3.3.41 Plasma Salicylate (Log Scale) vs Time For SSA:DOCA Coprecipitates.

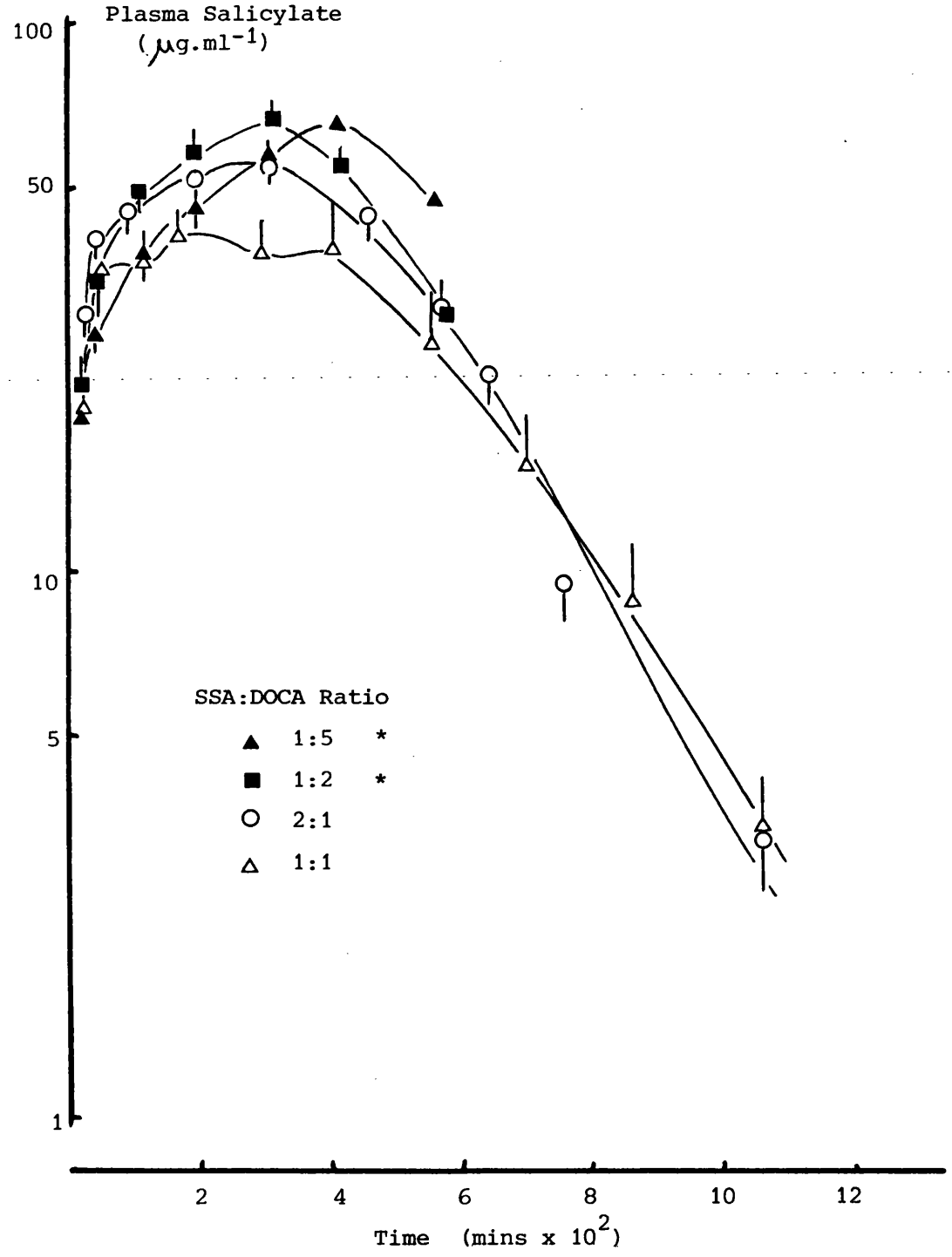


Fig. 3.3.42 Plasma Salicylate (Log Scale) vs Time For SSA:DOCA Admixtures.

* Truncated Due To Termination Of Experiment.

SSA:DOCA Ratio	SSA	2:1 COP	2:1 ADM	1:1 COP
2:1 COP	2.796 N = 12			
2:1 ADM	1.515 N = 12	1.047 N = 12		
1:1 COP	3.668 N = 12	1.509 N = 12	2.891 N = 12	
1:1 ADM	2.317 N = 11	0.312 N = 11	1.092 N = 11	1.464 N = 11

For N = 11 t_{tab} = 2.262

N = 12 t_{tab} = 2.228

p' = 0.05

Table 3.3.46 Student 't' Test Comparison Between Elimination Data For SSA:DOCA 2:1 and 1:1 Coprecipitates And Admixtures With SSA (75 - 105μm) Alone.

CHAPTER FOUR

DISCUSSION

Salicylsalicylic acid (SSA) was chosen as a model for this investigation for the reasons previously outlined. Firstly it seemed likely that its therapeutic efficiency would be limited by its poor solubility and secondly, that an improvement in the bioavailability of the drug by formulatory manoeuvres would provide an analgesic and anti-inflammatory salicylate that did not appear to produce extensive gastro-intestinal blood loss and was sufficiently prolonged in its action to provide alleviation of early morning stiffness. It was further hoped that rigorous examination of the physical, chemical and dissolution characteristics of this drug would also aid in an understanding of the relative importance of the different properties of other drugs known to exhibit incomplete bioavailability.

The findings of these investigations will be discussed in relation to basic pharmacokinetic studies on the drug in human volunteers and in rats. However, it is necessary to firstly consider some of the physical and chemical properties of SSA which, although they are not incorporated into the ensuing discussion at every opportunity, are fundamental to a full comprehension of the in vitro and in vivo data.

4.1 PHYSICAL CHARACTERISATION OF SSA

The original sample of SSA provided by A. B. Bofors was examined for its purity, crystallinity and particle size distribution in Sections 2.3.2, 2.3.5 and 2.4 respectively. The absence of any salicylic acid and other impurities, as shown by thin layer chromatography, suggests that the techniques of SSA separation

and purification during manufacture had successfully removed salicylic acid impurities, and that the material had not undergone any detectable hydrolysis prior to use.

The melting points determined in this study were higher than those ranging from 135-149^{o} quoted in the literature (See Table 2.3.4.1), although there was no evidence of a chronological sequence in these values that could be associated with continued improvement in production methods. The differences could result from varying techniques of determination although this is improbable since this study gave indistinguishable results employing the two methods of capillary tube (152^{o}) and the hot bench (152-153^{o}). Melting points can, however, be expected to decrease with the presence of impurities and the variety in the literature values may be attributable to the presence of different amounts of salicylic acid and/or other impurities. Subsequent to the quality control data provided for SSA (Bofors, 1971) the company have stated, in a private communication, that increased proficiencies in manufacturing methods since that date have improved the quality of the material such that the majority of the cited criteria were superceded. Since the bulk material supplied was manufactured in 1973 this may account for the higher melting point and the absence of salicylic acid.

The particle size of the material provided exhibited a logarithmically normal distribution (Fig. 2.4.3) that is characteristic of material where particle size reduction has been achieved by milling, grinding and other comminutive

processes (Herdan, 1960). However, the two data points for the lower particle size fractions deviated from the linearity of the log. normal plot: these two points are those of 30-38 and 38-50 μm and were obtained using micropore sieves that are known to have round apertures whereas all other sieve fractions were obtained using British Standard wire mesh sieves that have square apertures. Since the determination of particle size distribution by sieving depends on the probability of particles passing through a sieve of defined shape then the differences in the shape of the apertures for the two types of sieve used could account for the anomalous results derived using micropore sieves.

4.2 CHEMICAL CHARACTERISATION OF SSA

Determinations of SSA solubility in various solvents as a function of temperature (See 2.6) did not provide van't Hoff plots that were linear and hence amenable to thermodynamic examination (See Fig. 2.6.2). However, an interesting feature of these results shown in Fig. 2.6.2 is that SSA solubility exhibited a greater temperature-related change in 0.1N HCl (with or without 1% w/v ethanol) than in distilled water (with or without 1% w/v ethanol), since the curves converged at high temperatures. A probable explanation for these observations may lie in the fact that the ionisation constant (pKa) for carboxylic acids is related to temperature in a parabolic manner, with a maximum value occurring at 20-25^{o} (Albert and Sergeant, 1971). If the assumption is made that this is also true for SSA, then the observed pKa values of 3.45 and 9.83 (See Table 2.7.2) will decrease over the temperature range at

which solubility was determined. Thus the relative change in the proportions of ionised to unionised SSA as the temperature is raised will be more significant in 0.1N HCl at pH 1.0 than in water where a saturated solution of SSA has a pH very close to its pKa.

Another possible factor is that of drug stability since if a significant amount of salicylic acid is present it could interfere with the specificity of the SSA assay. This is unlikely since under conditions of high agitation intensity saturation solubility was achieved within 45 minutes whereas at pH 3.12 at 70° the $t_{10\%}$ for SSA hydrolysis is 50 minutes (calculated using the rate constant interpolated from the Arrhenius plot; Fig. 2.9.4). Therefore, even though hydrolysis may have occurred the salicylic acid liberated would not have significantly interfered with the SSA assay.

The potentiometric determination of the ionisation constants of SSA resulted in the production of a titration curve that deviated from being truely sigmoid in shape (See Fig. 2.7.1). Although the ultraviolet spectrophotometric method provided a sensitive technique for pKa measurement, some of the factors contributing to the deviation from the sigmoid shape of the titration curve warrant discussion.

The potentiometric determination of ionisation constants, although capable of being both rapid and accurate is prone to errors associated with temperature fluctuations affecting electrode response, the presence of carbon dioxide in the

alkali titre and low drug solubility. The first two criteria are readily controlled by the respective use of a constant temperature environment and by the preparation and maintenance of CO_2-free potassium or sodium hydroxide. However, drug solubility can place severe restrictions on the accuracy of this method since it has been suggested that a 0.01M drug concentration is an optimum for accurate pKa values without incurring the necessity for activity corrections (Albert and Sergeant, 1971). While recommending the use of 0.01M drug solutions these authors, based on their own experience, indicated that more dilute solutions could be used provided the negative logarithm of their molar concentration was not less than the expected pKa. Thus, a 0.0001M solution of SSA would be unsuitable if the pKa is less than 4. SSA solubility at 25^{o} (approximately 100 μg.ml.$^{-1}$, or 0.0004M; taken from Fig. 2.6.2) is bordering on the limits of this recommended dilution and the accuracy of the value obtained might be poor and imprecise since no definite neutralization point could be found (See Fig. 2.7.1). The use of a saturated solution is in itself another possible cause of error because a fall in the temperature of the solution after its preparation would result in precipitation of the drug. If this occurs prior to the commencement of the titration then any precipitated material would redissolve and produce a shallowing of, or a shoulder on the potentiometric curve. Even though no obvious signs of precipitation were seen the possibility of its occurrence and the formation of submicron crystals cannot be ruled out.

A further contributory factor could be that during the determination 2 molar equivalents of salicylic acid are formed by SSA hydrolysis, these would require a greater volume of titre for their neutralization thereby shallowing the potentiometric curve. This is unlikely since the experiments were performed within half an hour whereas the $t_{10\%}$ at 25^{o} in solution at pH 8.15 is 700 minutes (k value interpolated from Fig. 2.9.4).

It is probable therefore that the inaccuracy of the potentiometric method was a consequence of low drug concentration and possible precipitation. Furthermore it is unlikely that this method would have been able to describe the ionisation of the phenolic-hydroxyl group without using a stronger alkali titre which would have undoubtedly increased the likelihood of significant SSA hydrolysis.

4.3 BIOPHARMACEUTICAL CONSIDERATIONS RELEVANT TO SSA

With drugs where absorption is not permeability-limited it is believed that the rate of absorption is dictated by the concentration of the drug present in solution at the membrane surface in the non-ionised, lipophilic form. The degree of ionisation under in vivo conditions (determined by the Henderson-Hasselbalch equation(8)) is a function of the gastro-intestinal pH and drug pKa. SSA has been shown to possess two ionisation constants and to exist as three possible chemical species. pK_1 and pK_2 values of 3.45 and 9.83, respectively, have been determined in this study (See 2.7.2). Since the environmental pH of any physiological fluid will

rarely exceed pH 8.0, the bi-ionic species of SSA will not be found *in vivo* to any significant extent. However, under the acidic conditions prevailing in the stomach (pH 0.6-2.5) SSA would be present predominantly as the non-ionised moiety (99.6% at pH 1.0) and, as the intralumenal pH increased through the small into the large intestine, then the mono-ionic species would become predominant; ie at pH 7.4 SSA would be approximately 99.9% as the mono-ion.

The octanol/0.1N HCl partition coefficient (See 2.8) showed the non-ionised species of SSA to be freely lipid soluble at 37^{o}; K = 44.6. It could therefore be anticipated that the drug would be readily absorbed from solution in the stomach. Furthermore, as the pH increases along the gastro-intestinal tract then, according to strict interpretation of the pH-partition hypothesis, absorption would be expected to decrease due to a reduction in the prevalence of the absorbable moiety. This assumes that changes in the surface area of the various regions of the gut will not be a determining factor in the overall rate and extent of absorption and clearly this will not be the case (See 1). It can be expected, therefore, that SSA absorption, like that of salicylic acid (Schanker et al, 1957; 1958), would occur throughout the entire length of the alimentary tract provided that some fraction of the drug, however small, is present as the non-ionised form. Absorption would then only be limited by a slow or delayed dissolution and other formulatory influences.

Following characterisation of the dissolution apparatus, studies

on SSA show that the dissolution rate in 0.1N HCl containing $5x10^{-3}$% w/v polysorbate 80 at 37° is a function of particle size, manifested as a linear relationship with BET surface area (Fig. 2.11.4). The unexpectedly low dissolution rate of the micronized material may be ascribed to a decrease in the effective surface area for dissolution resulting from the formation of aggregates that were observed to persist for the duration of the experiments despite the incorporation of a wetting agent into the dissolution medium. Similar effects have been observed for the dissolution of griseofulvin and glutethamide by Lin et al (1968) who put forward two possible explanations for this effect. Firstly, they suggested that adsorption of air resulting from jet-mill micronization techniques might ultimately increase the hydrophobic nature of the powder and, secondly, that an increase in the interparticulate binding force might result from an electrostatic charge being imparted to the powder particles. This latter effect is the more likely since these same authors reported an increase in the electrostatic charge of powders to be a consequence of increasing surface area through particle size reduction.

It may have been possible, however, to overcome the formation of aggregates by increasing the polysorbate 80 concentration and/or the agitation intensity within the dissolution vessel, although these were not investigated. Any possible interference by salicylic acid resulting from SSA hydrolysis that occurred during the dissolution studies was negligible over the 2-6 hour period employed; as at 50° the $t_{10\%}$ in

0.1N HCl is approximately 42 hours, ($k = 7x10^{-7}sec^{-1}$, obtained by interpolation from Fig. 2.9.3); therefore after 6 hours at 50^{o} 2% hydrolysis would have occurred, a value which would be reduced at 37^{o}, the temperature used for dissolution rate determinations.

4.4 BIOPHARMACEUTICAL STUDIES

Since in all studies SSA was administered either as a solution, suspension or in capsules some comment is required both on the stability of the drug in these formulations and in the alimentary fluids prior to its absorption.

4.4.1 DEGRADATION OF SSA AND SSA FORMULATIONS

All aqueous dosage forms were administered within 15 minutes of their preparation; therefore the extent of hydrolysis within that period can be considered to be negligible. The stability studies reported in this thesis give no direct information regarding the stability of SSA powders over extended periods of time, except that in the complete absence of water vapour, hydrolysis will not occur. This is a difficult situation to attain and it can be assumed that SSA, like aspirin, will gradually degrade if stored in the presence of water vapour. Hydrolysis on storage has been demonstrated by Hanzlik and Presho (1925) who showed a highly significant positive reaction with ferric reagent for SSA that had stood in the laboratory for two years, whereas a freshly prepared sample showed no colour change at all. These results, although purely qualitative, are indicative of significant degradation and for this reason SSA and all SSA formulations were stored

in closed jars in a desiccator over phosphorous pentoxide.

4.4.2 DEGRADATION OF SSA IN VIVO

The concentration and time course of salicylate in the blood will be dependent upon two interacting factors: the rate of absorption and the rate of elimination. SSA absorption as the unchanged drug may be influenced by the extent of its hydrolysis within the gastro-intestinal lumen and the bioavailability would be severely reduced if appreciable in vivo degradation occurs. This has been shown for digoxin (Gault et al 1977) and is known to occur for aspirin (Levy and Angelino, 1968). However, the relatively slow rate of SSA hydrolysis under alimentary conditions cannot be expected to be a significant factor in determining the rate or extent of the appearance of salicylate in the plasma. Hanzlik and Presho (1925) have shown that 1.5% of a solution of SSA is hydrolysed after 24 hours at pH 6.0 and 38^{o} and that both 'pancreatine' and 'fresh beef bile' had a protective effect, retarding the liberation of salicylic acid. The conclusions of these authors were that the extent of intralumenal hydrolysis was negligible and although this is supported by the studies in this thesis it does not necessarily imply that the drug appearing in the systemic circulation is wholly unchanged since enzyme-mediated metabolism may occur during its passage across the gastro-intestinal mucosa, through the liver or within the vascular fluids of distribution. Transmucosal metabolism has been demonstrated for other salicylate esters such as Benorylate (Humphreys and Smy, 1975) and aspirin (Levy and Angelino, 1968). Similarly transmucosal conjugation of benzoic

acid with glycine (Strahl and Barr, 1971) and Salicylamide conjugation with glucuronic acid (Barr and Riegelman, 1970) further imply that metabolism may occur during the passage of SSA across the intestinal barriers.

Some drugs, ie aspirin (Harris and Riegelman, 1969) and lignocaine (Boyes et al, 1970), although well absorbed from the gastro-intestinal tract, exhibit poor systemic availability of the unchanged drug due to considerable metabolism occurring during passage through the liver. Such first pass effects for aspirin have been shown to account for a 32% loss of the drug following its oral absorption (Rowland et al, 1972) and, together with vascular and extravascular esterase hydrolysis, may account for its rapid elimination and the low amount of unchanged aspirin (1-2%) found in the urine (Cummings and King, 1966). In view of these observations for aspirin, the indications are that SSA hepatic clearance, vascular and extravascular degradation and elimination are less rapid than for aspirin and could provide an explanation for the prolonged salicylate levels in the blood and the presence of approximately 10% of unchanged drug in the urine following its oral administration (Hanzlik and Presho, 1925; Nordqvist et al, 1965).

4.5 SOLUBILITY AND IN VIVO PRECIPITATION

The initial urinary excretion studies in man demonstrated that SSA absorption from solution was rapid, albeit incomplete, and that the inter-subject variation in the apparent first order elimination rate constants was low (coefficient of variation =

4.4%). However, when the same dose was given in capsules containing 150-180 μm particle size material the urinary elimination rates for the same group of subjects showed greater inter-subject variation (coefficient of variation = 11.1%) even though the extents of salicylate absorption from both formulations were not significantly different, 55±1 and 57±3% dose for the solution and capsule respectively, values taken from Table 3.2.6 .

The equal, albeit incomplete, bioavailability of SSA following its oral administration in solution and capsules could be due to permeability - or dissolution rate-limited absorption. The latter may be a consequence of precipitation of the drug from solution on the one hand and incomplete dissolution from the solid material on the other. Permeability-limited absorption can be discounted for two reasons. Firstly there is no reported evidence of such behaviour for other salicylates, and secondly, although rat studies with SSA have shown incomplete absorption from solution, 100% bioavailability was observed for the SSA:CA 1:1 coprecipitate and admixture (See 3.3.17). This latter effect cannot be solely associated with bile salt induced changes in membrane permeability, since other SSA:CA and SSA:DOCA systems did not show similar effects, and was attributed to an increased dissolution rate shown to be peculiar to the SSA:CA 1:1 molar ratio formulations (See 2.13.5).

The proposed argument for incomplete absorption of SSA from solution is the occurrence of precipitation of the drug in the

gastric fluids. Such an argument is based on recrystallization studies carried out *in vitro* (See 2.10), where SSA was observed to precipitate from solutions of its sodium salt upon entering simulated gastric environments. Precipitates formed in human gastric juice and simulated gastric juice were of much smaller size than those produced in 0.1N HCl under identical conditions (See Fig. 2.10.3). These results for SSA and others reported in the literature for phenobarbitone sodium (Finholt and Solvang, 1968) . . suggest that some component of the gastric fluids may have a mediating effect on crystal growth *in vivo*. The components most likely to fill this role are the colloidal mucopolysaccharides and glycoproteins which comprise the mucin present in the gastric environment. This inhibitory effect of colloids and high molecular weight polymers is commonly used to retard and even prevent crystal growth and polymorphic transition in suspensions containing crystalline and/or amorphous substances (Mullins and Macek, 1960; Corrigan and Timoney, 1974; Stupak et al, 1974; Migazaki et al, 1976; Simonelli et al, 1970). Indeed, the majority of these authors have shown PVP to be one of the most effective agents for preventing crystal growth and it was therefore investigated with respect to its influence on SSA precipitation in the *in vitro* simulated system. Fig. 2.10.3 shows that SSA crystals produced in the presence of 0.1 and 2% w/v PVP were much fewer and angular than those for either the gastric fluid or 0.1N HCl. These observations for PVP suggest that *in vivo* precipitation of SSA may be prevented by the incorporation of PVP into the formulated solution, thereby increasing its absorption, although this was not investigated *in vivo*.

The low coefficient of variation (4.4%) for the first order elimination rate constants obtained from the urinary excretion data for the solution may possibly be explained by the fact that all subjects were observing a strict experimental protocol, under which variations in gastric fluid volume, contents, pH, movement etc would be minimal. Thus if precipitation has occurred the variation in the extent and time course of recrystallization and subsequent redissolution would also be minimal, whereas the higher inter-subject variation shown for the 150-180 μm particle size material (coefficient of variation 11.1%) is indicative of an erratic rate of absorption and probably reflects incomplete and irregular dissolution of the larger particles.

Possible polymorphic changes in the in vivo precipitates should be considered as a further complicating factor since if formed they may have different intrinsic solubilities or dissolution rates than the parent material. However, X-ray diffraction studies on the SSA precipitates formed in 0.1N HCl, human and simulated gastric juice and PVP solutions did not show any differences in their X-ray diffraction pattern from the original material and from each other (See Fig. 2.10.4). This suggests that although crystal growth in each system is affected by the medium the resultant crystals differ only in their habit and not their crystalline structure.

It has previously been proposed that drugs possessing a narrow therapeutic index and dissolution rate-limited absorption, eg digoxin, will manifest therapeutic inconsistencies associated

with variable absorption. For compounds, such as the salicylates, where the therapeutic index is high, problems of toxicity will not be as acute; indeed, for chronic rheumatic conditions where prolonged administration is necessary, small deviations in bioavailability will induce little change in the overall therapeutic efficiency. In spite of this, it is desirable that as complete and reproducible an absorption as possible should occur for all drugs (except those exerting local effects within the gut). The need for this is exemplified by the barbiturates which are known to have a narrow therapeutic index and which exhibit variable absorption that can often instigate the self-administration of a second and toxic dose following an initial one ineffective in its sedative action due to a delayed absorption.

The possibility of gastro-intestinal precipitation may be of further consequence when considering a drug solution as a reference for the assessment of oral bioavailability in the absence of an intravenous standard. All guide lines for measurement of drug availability advocate the oral administration of a solution where drug solubility, toxicity or ethical factors prohibit its intravenous injection (eg. Notari, 1975; Wagner, 1975). The studies with SSA and other sparingly soluble drugs have shown that precipitation can occur following their oral administration in solution as a soluble salt which may result in an unexpectedly low and incomplete absorption. It can be anticipated therefore, that other compounds may exhibit similar behaviour and that the rate and extent of their in vivo precipitation may be greatly influenced

by the gastro-intestinal environment such that, where significant precipitation occurs, a large inter-subject variation in absorption can be expected. If this is the case, then the oral bioavailability standard may be better served by administration of a homogeneous suspension of a known particle size distribution. One such case is that for the oral solution of reserpine employed by Stupak and Bates (1973) as a standard for estimating the bioavailability of reserpine coprecipitates. They attributed an unexpectedly low availability of the drug in solution to its precipitation in the intestine (reserpine being a basic drug). This phenomenon may also provide a further interpretation of the variable human absorption rates of aspirin from solution, over and above those of physiological origin postulated by Rowland et al (1972).

4.6 SALIVA AND PLASMA CORRELATION STUDIES

Studies designed to demonstrate a correlation in salicylate concentrations in plasma and saliva produced data that showed a time related change in their concentration ratios. (See Figs. 3.2.11, 12 and 14). This change was associated with the fact that the times of peak salicylate concentration for plasma and saliva were not identical and that a stable correlation between the two compartments was only achieved during the elimination phase, ie. post peak.

A true correlation between salicylate concentrations in the saliva and plasma will only be obtained if the concentrations measured in each compartment represent the fraction of the drug that is freely available to be transferred across the

glandular epithelium. The protein-bound fraction within the plasma is not able to freely pass into the saliva so that any displacement of that bound fraction during assay would incur an erroneously high and perhaps variable value for transferable salicylate. The protein binding of salicylate will be subject to alteration for a variety of reasons. The relative proportions of unchanged drug and its various metabolites will be altered by continuing absorptive, metabolic and excretory processes, and, if concentrations permit may exhibit competition for the albumin binding sites since each moiety will have different binding characteristics. Furthermore, since the extent of partitioning into saliva will be a reflection of the extent of protein binding the experimentally measured concentrations in saliva and plasma could be in error if, during the assay bound material, or a fraction of it, is displaced in such a manner that the displacement is preferential for a particular salicylate(s) present. It would also seem probable that binding of SSA and its metabolites may be dose/concentration-related since unbound plasma salicylate has been shown to increase with dose (Smith et al, 1946) and urinary elimination of salicylate decreases with an increase in plasma protein concentration (Hollister and Levy, 1965).

It would therefore, appear that the time related change in the saliva:plasma concentration ratio could be associated with alterations in plasma protein binding, particularly, those that may occur during storage of experimentally obtained plasma samples at -15°, their acidification with 4N HCl or during the extraction process into ether. In view of this more

information is needed before saliva can be inferentially used to fully describe salicylate disposition following administration of SSA. The most obvious answer would be to specifically determine the unbound SSA in both physiological compartments.

The comparability of salicylate elimination rates, determined for the higher dose from saliva and urinary excretion data, has been established for SSA (See 3.2.6) and for sodium salicylate (Page et al, 1974), and indicates that saliva, while prone to contamination from certain food-stuffs and dosage forms etc, is capable of providing an adequate description of salicylate elimination. Furthermore, providing the complete elucidation of the problems associated with plasma assays and protein binding behaviour has been achieved, saliva will adequately describe drug absorption; bioavailability data could then be calculated with some degree of confidence. Regarding this last point, AUC data for saliva following intravenous drug administration would provide an absolute availability standard for comparison.

Following oral administration of aspirin to man some subjects displayed secondary and even tertiary peaks on the saliva salicylate profiles at times where meals had been taken (See Fig. 3.2). The phenomenon of changes in drug absorption in the presence of food and under fasting conditions is well recognised and reviewed elsewhere (Wagner, 1971; Notari, 1975; Gibaldi, 1971; among others). Enhancement of drug absorption associated with food intake can result from several, often

interacting, physiological and physicochemical processes. Physiological considerations include changes in gastro-intestinal motility, fluid secretions, bile flow and mesenteric blood flow. Probably the major influences are those of gastric emptying, resulting in unabsorbed drug moving into the small intestine where an increase in pH will promote dissolution and an increase in mesenteric blood flow and absorptive surface area will enhance the rate of absorption. Elementary Pavlovian reflex mechanisms will raise saliva flow which may promote salicylate transfer into the saliva and thereby increase the concentration, albeit transiently. However, salicylate transfer into the parotid salivary glands of the dog has been shown not to be influenced by saliva flow rate (Borzelleca and Putney, 1970). Furthermore, such an effect would have been observed in all subjects and would have resulted in a much greater intra- and inter-subject variation than was observed since the salivating ability of each subject varied. Although aspirin was given in solution in 300, 600, and 900 mg doses, *in vivo* precipitation cannot be ruled out and since the mid-profile peaks were only manifested by subjects receiving the two higher doses they could be attributed to a promoted redissolution of any precipitated material by the mechanisms previously discussed.

Urinary excretion studies in man presented in this thesis are conflicting in that for an oral dose of 60 mg SSA given in solutions and in capsules the percentages of the dose excreted after 24 hours were 55±1% and 57±3% respectively whereas, at a higher dose of 600 mg, given as micronized material in capsules,

a mean value of 32±4% was obtained (See 3.2.3 and 3.2.5). Although at the lower dose the observed percentage excreted agrees with those of 52% and 55-75% quoted in the literature for SSA doses of 1-12 g (Hanzlik and Presho, 1925; Bastide, 1955), no explanation, other than a genuine decrease in absorption for the encapsulated micronized material at the higher dose can be offered. This conclusion is based on the observations of Newton and Rowley (1970) who filled different particle size ranges of a poorly soluble drug into capsules at a known porosity. They measured the dissolution rate of the drug and found that at any given porosity the faster dissolution rate was obtained with the larger particle size material. They attributed this to a reduction in the effective surface area of the finer particles available to the dissolution medium and associated it with the packing of the capsules. Similarly, Aguiar et al. (1967) have studied this phenomenon and showed that the greater the compaction inside the capsule the poorer the dissolution rate; this problem was more significant with poorly soluble drugs. The reduced bioavailability of SSA from the 600 mg dose in comparison to the 60 mg dose could, therefore, be explained in terms of the extent of packing and particle size since the larger dose consisted of micronized material firmly packed into two Size 0 hard gelatin capsules whereas the smaller 60 mg dose of 150-180 μm particle size was loosely contained in a single Size 0 capsule.

Elimination half lives for total salicylate have been reported in the literature for SSA following its oral administration.

In a study using a single subject urinary excretion data represented as a 'Sigma minus' plot gave a $t_{\frac{1}{2}}$ of 4.55 hours (Bastide, 1955) and a mean elimination half life of 7.8 hours (range 6.54-9.63) has been calculated using plasma salicylate concentration data from 9 subjects each receiving an oral dose of 500 mg SSA (Nordqvist et al, 1965). Both studies suggest that the half lives of 4.95 and 5.77 hours for LS and NS calculated from plasma data obtained in this work represent the true elimination rate although this cannot be categorically stated since the data points used in their determination were not unequivocally shown to completely describe the terminal elimination phase.

4.7 RAT STUDIES

Studies in the rat with SSA were carried out initially to describe the influences of route of administration, dose and oral vehicle on the disposition of salicylate, and subsequently to investigate the influence of particle size and coprecipitation of SSA with PVP, CA and DOCA on the rate and extent of absorption.

The assessment of drug bioavailability was achieved by comparing the AUC for the test dosage form with those for either an equivalent intravenous or oral standard, to give absolute or relative values respectively. Before reviewing the results and drawing conclusions from these studies several aspects of the experimental protocol and treatment of the data warrant discussion.

4.7.1 THE EXPERIMENTAL PROTOCOL

The number of blood samples taken from each animal over the course of a single experiment can account for up to a 9 ml volume. If this was removed as a single entity the haemodynamic condition of the animal would be dramatically affected, resulting in hypotension, changes in blood distribution and acute distress. Assuming a 500 g body weight, the total blood volume can be calculated to be approximately 27 ml (ie. 54 $ml.kg.^{-1}$: a mean figure taken from Altman, 1961). Therefore over a 24 hour period up to one third of the original blood volume may have been removed. However, although the haematocrit can be expected to decrease the total plasma volume and protein concentration may not alter significantly since normal physiological control mechanisms will rapidly replace body water by increasing fluid intake and, replace protein by promotion of the appropriate liver enzyme systems.

Comparing various haematological parameters of blood samples, obtained by cardiac puncture and from the tail of the rat, Upton and Morgan (1975) have shown that tail blood gave consistently higher haematocrit and haemoglobin values. They further showed that manually-restrained animals gave higher levels of haemoglobin, plasma protein and haematocrit than those animals restrained by ether (gaseous), pentobarbitone sodium (5 mg. 100 g^{-1}, ip) or fentanyl-droperidol (0.1 ml 100 g^{-1}, im). The conclusions drawn from this suggest that the stress induced by manual restraint reduces plasma water and that the site of sampling will influence the haematocrit and haemoglobin levels. Unfortunately these authors did not

investigate the influence of repeated sampling from the tail on these parameters, which can be expected to decrease in a manner dictated by the frequency and size of sampling and may, therefore, induce complicating changes in drug disposition. Decreases in blood volume may cause secondary compensatory alterations in the distribution of inter- and extra-cellular fluids to an extent that pharmacokinetic compartmental volumes of distribution may change in a time-related fashion. An example of such a change is an increase in the volume of distribution of lignocaine in the rhesus monkey induced by raising cardiac output with isoprenaline, and a reduction in the volume following haemorrhage (Benowitz et al, 1974). From these considerations it would be expected that changes in salicylate disposition would result from alteration of the integrity of the blood. However, a method for repeated blood sampling in the conscious rat has been reported (Agrelo and Miliozzi, 1974) where replacement of six 1 ml blood samples (taken from 100-250 g rats) with whole blood from a donor animal or with saline did not produce any significant change in the disposition of intravenously-administered quinidine sulphate. Both profiles were indistinguishable from each other and from a profile composed of single samples, ie, one sample per animal only.

Since the same experimental protocol was adopted for each of the studies on SSA the changes in blood integrity, if significant, can be expected to be similar and will not distract from their comparability. There was one study, however, where in an attempt to describe the compartmental distribution of

salicylate following a low (2.5 mg.kg.$^{-1}$) intravenous dose, fifteen blood samples were taken over a five hour period. It is interesting to note that the apparent first order elimination rate constants calculated from the data (See Fig. 3.3.4 and Table 3.3.5) were significantly higher than those for the other intravenous doses, and may, therefore, be a direct consequence of changes in salicylate protein binding and compartmental distribution resulting from an excessive reduction in plasma protein concentration and body water. Both of these parameters can be expected to promote the renal clearance of the drug and could account for the increased K_{el} values.

4.7.2 TREATMENT OF PLASMA LEVEL DATA

The two main parameters used to assess the influences of a formulatory change on SSA absorption have been the area under the curve (AUC) of the plasma level profile and the apparent first order elimination rate constant (K_{el}). When drug disposition can be accurately described by a simple compartmental open model where all transfer processes are first order then the AUC and K_{el} values can be calculated using the appropriate differential equation(s). In the situation where the capacity-limited and first order processes occur in conjunction with an inadequately-defined compartmental model and, where no suitable computerized analytical method is available, then AUC and K_{el} values for a specific dose can be calculated by graphical means. The AUC and K_{el} data presented in this thesis were consequently calculated from the profiles for individual animals and are presented with their mean and standard errors.

4.7.3 ELIMINATION RATE CONSTANTS

The number of points that describe the linear terminal phase of a plasma salicylate concentration (log scale) vs time plot will automatically determine the accuracy of any derived rate constants. In most cases this terminal phase was composed of 4-7 data points although in some instances there was no apparently linear section readily distinguishable. However, where K_{el} values are quoted at least three data points were used in their calculation. For some studies the points defining a linear section were obvious whereas for the greater proportion there was a large subjective element in deciding which points to include and which to exclude, particularly at the beginning of the terminal phase where the curvature of the zero order data on a log scale merges with the first order data or where absorption is still occurring.

All elimination rate constants (mean ± SE) obtained throughout these studies are collated in Table 4.1. Most of these values are similar and variance ratio analysis carried out for each series of studies show that salicylate elimination is not significantly influenced by formulation, dose or route of administration. Several exceptions do, however, exist (denoted by an asterisk in Table 4.1) and will be commented on separately.

-2.5 $mg.kg.^{-1}$, iv. A possible explanation for the increased rate of elimination observed in this study has already been discussed as part of the experimental protocol (See 4.7.1).

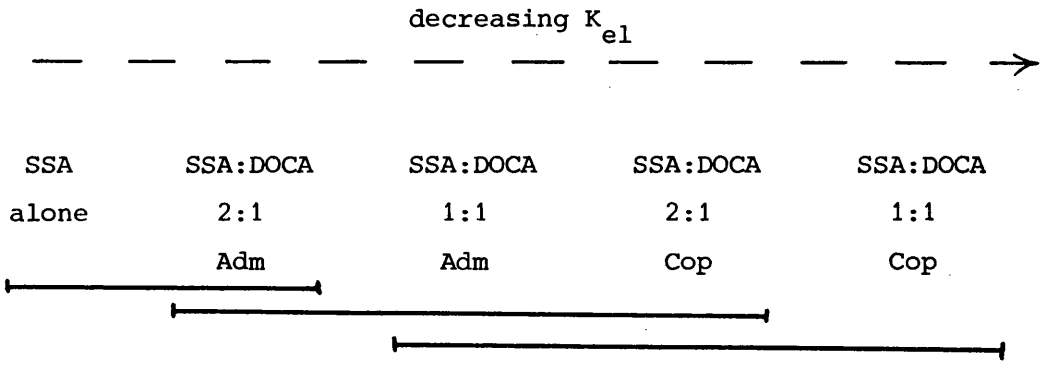
-150-180 μm particle size, 20 $mg.kg.^{-1}$, po. Although the

Dose (mg.kg.$^{-1}$)	Route	Formulation	K_{el} mean ± SE ($\times 10^{-3}$ min.$^{-1}$)
2.5	iv	Solution 25% Ethanol in Sørensens PO_4 pH 7.4	8.446 ± 1.103*
10	"	" " " "	5.370 ± 0.965
20	"	" " " "	5.805 ± 0.617
30	"	" " " "	6.236 ± 0.799
50	"	" " " "	6.374 ± 0.702
20	po	Solution	6.668 ± 0.242
"	"	75-105 µm in 4% PVA	5.520 ± 0.505
"	"	" in water	4.280 ± 0.367
100	"	" in 4% PVA	5.374 ± 0.096
50	"	" "	6.555 ± 0.506
10	"	" "	6.025 ± 0.090
20	"	Micronized "	4.239 ± 0.387
"	"	38-53 µm "	6.472 ± 1.274
"	"	53-75 µm "	5.100 ± 0.354
"	"	105-150 µm "	5.980 ± 0.409
"	"	150-180 µm "	3.227 ± 0.172*
"	"	SSA:PVP 2:1 COP "	5.360 ± 1.053
"	"	" 1:1 " "	5.471 ± 0.495
"	"	" 1:2 " "	4.637 ± 0.479
"	"	" 1:5 " "	5.620 ± 0.189
"	"	" 1:10 " "	5.418 ± 0.663
"	"	" 2:1 ADM "	4.535 ± 0.418
"	"	" 1:1 " "	5.416 ± 0.637
"	"	" 1:2 " "	5.819 ± 0.385
"	"	" 1:5 " "	5.638 ± 0.412
"	"	" 1:10 " "	5.487 ± 0.595
"	"	SSA:CA 2:1 COP "	5.902 ± 0.296
"	"	" 1:1 " "	4.461 ± 0.325
"	"	" 1:2 " "	5.712 ± 0.349
"	"	" 1:5 " "	4.441 ± 0.180
"	"	" 2:1 ADM "	5.010 ± 1.025
"	"	" 1:1 " "	5.298 ± 0.111
"	"	" 1:2 " "	5.824 ± 0.620
"	"	" 1:5 " "	5.061 ± 0.314
"	"	SSA:DOCA 2:1 COP "	3.975 ± 0.223*
"	"	" 1:1 COP "	3.512 ± 0.210*
"	"	" 2:1 ADM "	4.615 ± 0.318
"	"	" 1:1 " "	4.103 ± 0.345*

Table 4.1 Collated mean (± SE) Apparent First Order Elimination Rate Constants (K_{el}) From All Rat Studies

variance ratio analysis data given in Table 3.3.17 for the K_{el} values does not show a significant influence due to particle size, the associated variance is greater than that of animal origin and the Student 't' test analysis between particle size treatments (Table 3.3.15) shows the K_{el} values for the 150-180 μm material to be significantly less than for all other particle sizes. This difference is readily apparent by examination of Fig. 3.3.19. The probable explanation for the lower K_{el} value is also apparent from closer examination of the data points describing the terminal elimination phase for the larger particle size study. The terminal linear phase of the first order plot is ill-defined since the last samples plotted were taken well before the limits of the assay were reached and therefore describe salicylate disposition that may not necessarily be that for first order elimination. Even though, the variance ratio analysis suggested that the larger particle influences elimination, and the Student 't' test shows it to be significantly different from all other particle sizes, it cannot be inferred that the size of the material was directly responsible for the anomalous result. It should be pointed out, however, that in all the other cases where K_{el} values are given, the first order plots from which they were calculated had all reached the limits of the salicylate assay.

-SSA:DOCA 2:1 coprecipitate and 1:1 coprecipitate and admixture. Student's 't' test comparison between the SSA:DOCA systems and with SSA alone has been given in Table 3.3.46, from which the following decreasing rank-order can be defined, where the bars show no significant difference in K_{el} data



The influence of a large intestinal presence of bile acids is known to result in significant decreases in both electrolyte (K^+, Na^+, Cl^-, HCO_3^-) and water transport across the intestinal mucosa and it is also known that this effect is more severe with deoxycholic than cholic acid (Mekhjian and Phillips, 1970). The difference in effect between the two bile acids has been attributed, by the previous authors, to the fact that the deoxycholic acid absorption from the colon occurs at twice the rate of that for cholic acid. If this is so, then the bile acid could be present in toxic concentrations within the systemic circulation and changes in electrolyte and water flux observed for the intestinal epithelia may then occur in the nephron to such an extent that similar decreases in electrolyte and water transport ensue; ie, their passage into the urine decreases producing a reduction in urine flow and pH, thereby retarding salicylate elimination. Deoxycholic acid has been demonstrated to reduce the activity of the intestinal mucosal sodium- and potassium-ATPase (Gracey et al, 1973); consequently the renal activity of this enzyme can be expected to be reduced when the bile acid is present in toxic concentrations. Such a reduction in ATPase activity would decrease the active

tubular secretion of salicylate, especially since glycine conjugates are excreted via this mechanism (Schachter and Manis, 1958).

Another metabolic effect of deoxycholic acid on salicylate elimination is the possible competition between salicylate and DOCA for enzymatic conjugation with glycine. Salicylate concentrations in the urine account for up to 80% of a dose of sodium salicylate so that a reduction in the rate of its formation may be reflected by a reduction in the overall elimination rate constant.

One further feature of interest in Table 4.1 is the lack of effect of ethanol (used as a cosolvent for the intravenous studies) since it is known to promote drug elimination via its diuretic action. The total amount administered in the i.v. bolus was obviously insufficient to effect such a change.

4.7.4 AREA UNDER THE CURVE

Precise calculation of the area under the plasma level curve can only be achieved by integration (between t_0 and $t\infty$) of the appropriate differential equation(s) shown to completely describe the disposition of the drug. Previous comment has been made regarding the complexities of obtaining and solving this type of equation when parallel capacity-limited and first order processes occur (See 3.1) and it was therefore necessary to resort to using either gravimetric, planimetric or trapezoidal techniques for determining the area. The accuracy of these methods depends on there being sufficient data points to completely define the profile and errors in AUC

values calculated using these methods can be expected if the data points are themselves in error, or if significant sections of the profile are ill-defined. This last factor is particularly important for the absorptive phase following oral administration or the distributive and terminal phases for all routes. For precise and accurate values the terminal elimination phase should be extrapolated to infinite time although in the method employed in these studies the profiles were extrapolated to the time where the limits of the assay were reached.

The method of comparing AUC for intravenous and oral data to gain assessments of % bioavailability assumes that the plasma clearance is constant and that the area is a linear function of dose. Possible non-linearities in plasma clearance caused by excessive blood sampling have been mentioned and can be assumed to show little experiment-to-experiment variation since the experimental protocol was unaltered throughout. Furthermore, even though non-linear sections of the plasma profiles were observed after intravenous administration (and used to contruct a Lineweaver-Burk plot) their respective AUC values were shown to be linearly related to dose following both iv and oral administration (See Figs. 3.3.8 and 3.3.14 respectively).

4.8 INFLUENCE OF VEHICLE ON SSA ABSORPTION

SSA has been orally administered to the rat at a dose level of 20 $mg.kg.^{-1}$ in solution and as 75-105μm particle size material suspended in 4% PVA and water. Each of these vehicles produced different plasma salicylate profiles (See Figs. 3.3.9 and

3.3.11), the various features of which are given in Table 4.2.

Vehicle	Profile Peak Salicylate		K_{el} ($\times 10^{-3}$ min.$^{-1}$ $\pm$SE)	AUC (μg.ml.$^{-1}$ xmin.)	% Bioav (mean)
	Time (mins.)	Concentration (μg.ml.$^{-1}$)			
Solution	25 (115)	64 (66)	6.668$\pm$0.242	24257	54
4% PVA	100 (390)	54 (58)	5.520$\pm$0.505	40532	89
Water	90 (420)	48 (58)	4.280$\pm$0.367	37961	84

Table 4.2 Characteristics Of The Plasma Salicylate Profiles For SSA, 20 mg.kg.$^{-1}$ PO Given In Solution And Suspensions (75-105 μm) in 4% w/v PVA And Water. (Figures in parenthesis denote a secondary peak).

One common feature of all three profiles is the appearance of a second peak which probably occurs when SSA enters the small intestine where the larger absorptive surface area and higher intralumenal pH will enhance absorption. The initial peak is due to the gastric absorption of SSA present in solution and would account for the early peak of higher concentration shown when SSA is given wholly in solution. The secondary peak for this study could be attributed to *in vivo* precipitation with subsequent incomplete intestinal redissolution as well as to physiological changes previously mentioned. A longer time gap between the first and second peaks for the suspended

formulations would suggest that dissolution was the limiting factor rather than gastric emptying since the PVA and aqueous vehicles produced similar profiles.

The surprisingly low % bioavailability for the solution could not only be a reflection of in vivo precipitation but also an elimination rate that is in the upper range of values given in Table 4.1 since it could be expected that the buffer salts present in the solution would ultimately elevate urinary pH thereby promoting salicylate excretion. The presence of the potassium cation in the buffer solution may further inhibit SSA absorption as has been demonstrated to occur for salicylic acid in the everted rat intestine (Mayersohn and Gibaldi, 1970). These authors concluded that the reducing effect of K^+ (as opposed to Na^+) was due to an increase in the intestinal tissue fluid uptake which ultimately resulted in a decreased drug transfer rate. A similar conclusion for salicylate transfer has been made by Benet et al. (1971).

4.9 INFLUENCE OF DOSE

The influence of dose on salicylate profiles and AUC following intravenous administration has already been described (See 3.3.9.1).

Following oral administration of SSA, 75-105 μm particle size, at four dosage levels, several features of the resultant salicylate profiles (Fig. 3.3.13) other than AUC can be related to dose. Plots of the initial peak concentration and time against dose are given in Fig. 4.1 and both are shown to be

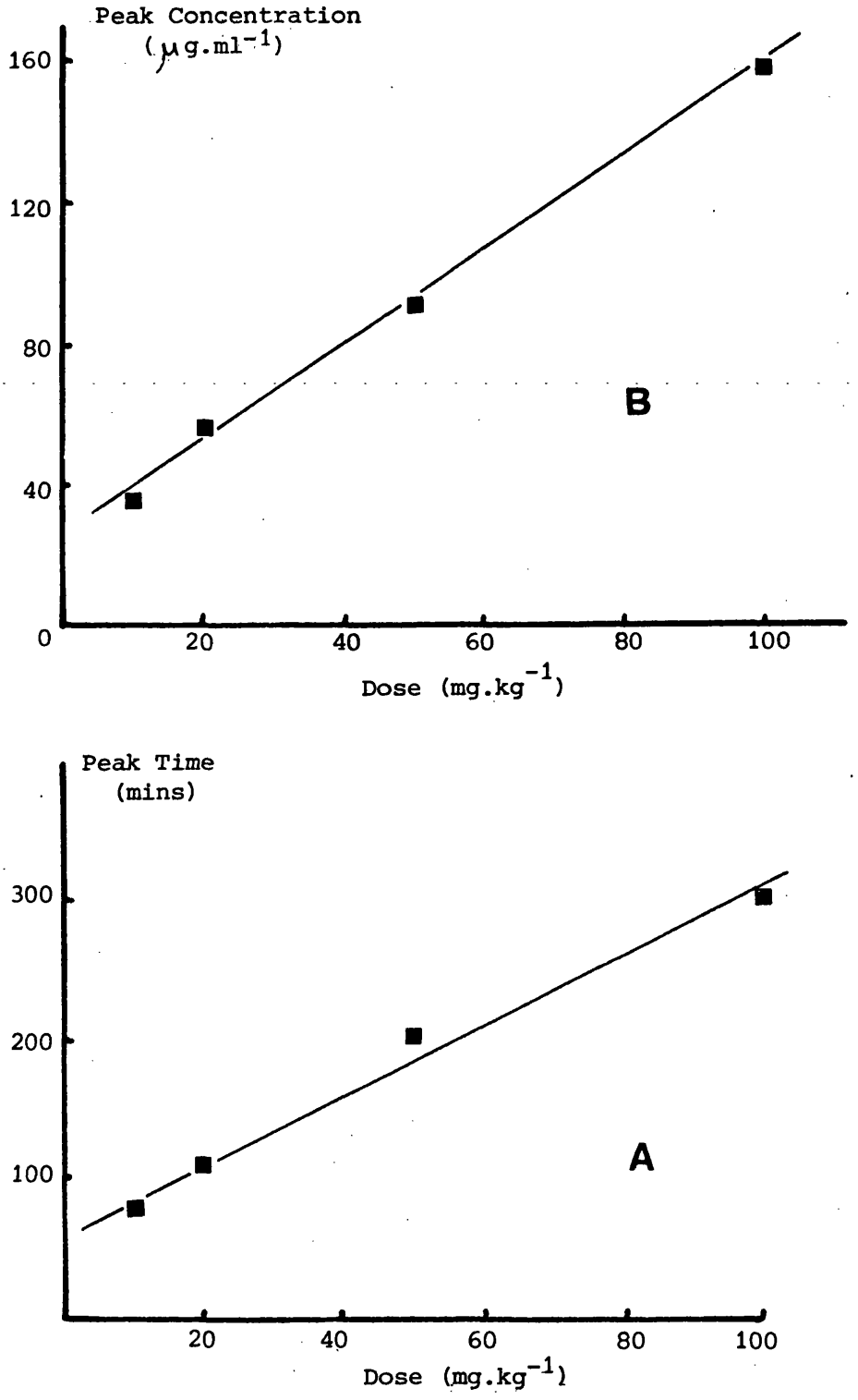


Fig. 4.1 Relationship Between Peak Time (A) And Peak Concentration (B) With Dose For Orally Administered SSA, 75-105 μm.

linearly related suggesting that these parameters can justifiably be used to assess changes in the rate of SSA absorption in the absence of a suitable mathematical approach. The peak times and concentrations were interpolated from Fig. 3.3.13, using the first peak if secondary ones were also present.

4.10 INFLUENCE OF PARTICLE SIZE

A correlation between the apparent first order dissolution rate constant and BET surface area has already been established (Fig. 2.11.4) and enforces the concept that SSA would exhibit dissolution rate-limited absorption. Such a relationship is indeed demonstrated in Fig. 4.2 where the initial peak time (log scale) is shown to be linearly related to BET surface area. The data used to construct Fig. 4.2 is given in Table 4.3 below.

Particle Size (μm)	Peak time (mins.)	Peak conc. ($\mu g.ml.^{-1}$)
Micron	25 (270)	45 (46)
38-53	55 (195)	52 (61)
53-75	70 (220)	51 (51)
75-105	105 (455)	54 (57)
105-150	120	51
150-180	240	53

Table 4.3 Peak Times And Concentrations For Plasma Salicylate Profiles Following Oral Administration Of SSA Of Varying Particle Sizes. (Numbers in parenthesis denote a second peak).

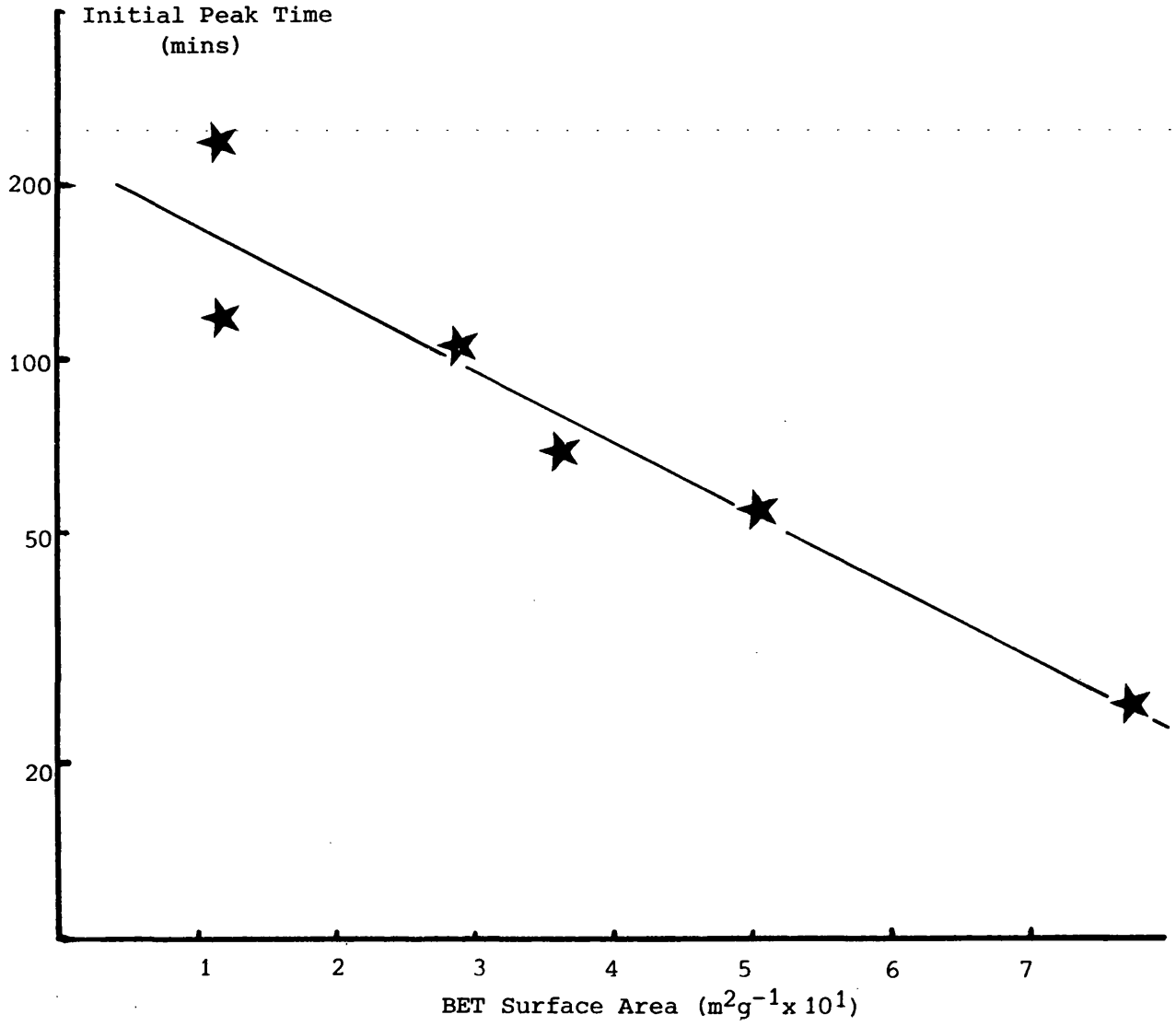


Fig. 4.2 Relationship Between BET Surface Area And Initial Peak Time (Log Scale) Following Oral Administration Of SSA Of Varying Particle Size Ranges. Dose 20 $mg.kg^{-1}$.

The peak salicylate concentration in the plasma does not show any obvious dependency on the particle size of the administered drug and the AUC data given in Table 3.3.12, although shown by the variance ratio test to be influenced by particle size, do not lend themselves to any logical explanation since they cannot be related to any other feature of their salicylate profiles nor to the percentage of the dose excreted determined by urinary excretion studies (See 3.3.14).

4.11 SSA BIOAVAILABILITY FROM COPRECIPITATES AND ADMIXTURES WITH POLYVINYLPYRROLIDONE (PVP)

Preliminary urinary excretion studies on SSA:PVP coprecipitates showed absorption from the 1:5 ratio to be significantly less than that for the other SSA:PVP ratios and for SSA alone. (The 1:10 ratio was not studied) (See 3.3.15). Subsequent plasma level studies have further shown the 1:5 SSA:PVP coprecipitate, as well as the 1:10 ratio, to exhibit a lower area under the curve in comparison to the pure drug and the 2:1, 1:1 and 1:2 systems. The bioavailability for each system was calculated using the mean AUC value for each in comparison to an equivalent intravenous and oral dose and is given in Table 4.4.

It is apparent that, even though X-ray diffraction studies have shown the SSA:PVP coprecipitates to be amorphous, their potential for improving SSA bioavailability was not realized in vivo. Ranking of the AUC data shows that for the 2:1 and 1:1 ratios the coprecipitates manifested a larger value than their corresponding admixture whereas for the 1:2, 1:5 and

SSA:PVP Ratio	Bioavailability (%)					
	SSA:PVP COPRECIPITATES			SSA:PVP ADMIXTURES		
	Mean AUC $\mu g.ml.^{-1}$ xmin	vs SSA iv	vs SSA po	Mean AUC $\mu g.ml.^{-1}$ xmin	vs SSA iv	vs SSA po
2:1	38954	85.8	96.1	33159	73.1	81.8
1:1	42139	92.9	103.9	40567	89.4	100.1
1:2	33886	74.7	83.6	40727	89.7	100.5
1:5	28692	63.22	70.8	29761	65.6	73.4
1:10	29406	64.8	72.6	38485	84.8	94.9

AUC reference iv 45383 $\mu g.ml.^{-1}$xmin (From Table 3.3.6)

AUC reference po 40532 $\mu g.ml.^{-1}$xmin (From Table 3.3.12)

Table 4.4 Bioavailability For SSA:PVP Coprecipitates And Admixtures Calculated From Mean AUC Data.

1:10 ratios the order was reversed. (Table 4.5).

SSA:PVP ratio	AUC ranking
2:1	COP > ADM
1:1	COP ≫ ADM
1:2	ADM ≫ COP
1:5	ADM > COP
1:10	ADM ≫ COP

Table 4.5 Ranking Of AUC Data For SSA:PVP Coprecipitates (COP) And Admixtures (ADM) For Various Drug:Polymer Ratios

A possible explanation for these results may be obtained by consideration of the extensive in vitro studies on sulphathiazole:PVP coprecipitates reported by Simonelli et al, (1969; 1976). Based on investigations designed to examine sulphathiazole and PVP release rates from dissolving coprecipitate discs and their transfer using cell diffusion studies, the authors came to a general conclusion that, provided drug:PVP coprecipitates are amorphous, it can be expected that with an increasing drug:PVP ratio the dissolution rate will increase up to a critical drug:PVP ratio after which it will fall. Even though their studies were performed using compressed discs the conclusions may nevertheless be applied to dissolution of particulate drug:PVP systems since the mechanisms of dissolution will be the same. The plot of AUC vs SSA:PVP ratio, given as

Fig. 4.3, does show a maximum bioavailability for the 1:1 coprecipitate with a subsequent decrease for the 1:2, 1:5 and 1:10 ratios; this suggests that the in vitro findings for the studies on sulphathiazole:PVP systems may provide a valid explanation for the results obtained in vivo for SSA:PVP coprecipitates. This is further enforced by the observed amorphous nature of all the SSA:PVP coprecipitates. Since coprecipitation with PVP reduced SSA bioavailability in every case, except for the 1:1 ratio, a detailed examination of their in vitro dissolution and solubility characteristics was not performed and may furnish a suggestion for further work.

The amorphous nature of the coprecipitates probably results from PVP being in sufficient concentration to form a net-like structure around the SSA molecule during solvent evaporation thereby preventing nucleation and the formation of a regular crystal lattice. This type of behaviour has been assigned to the inhibition of sulphathiazole crystal growth in the presence of PVP (Simonelli et al, 1970) and is commonly employed to stabilize and prevent polymorphic transition with suspensions of amorphous substances (Mullins and Macek, 1960). In all cases where X-ray diffraction has been employed drug coprecipitates with PVP have been shown to be amorphous; this has been attributed to be responsible for increased absorption although enhanced drug absorption has also been attributed to an increase in the surface area of the drug (ie. reduced particle size) produced during coprecipitate preparation (Bates et al 1969). See Section 1.4.3.

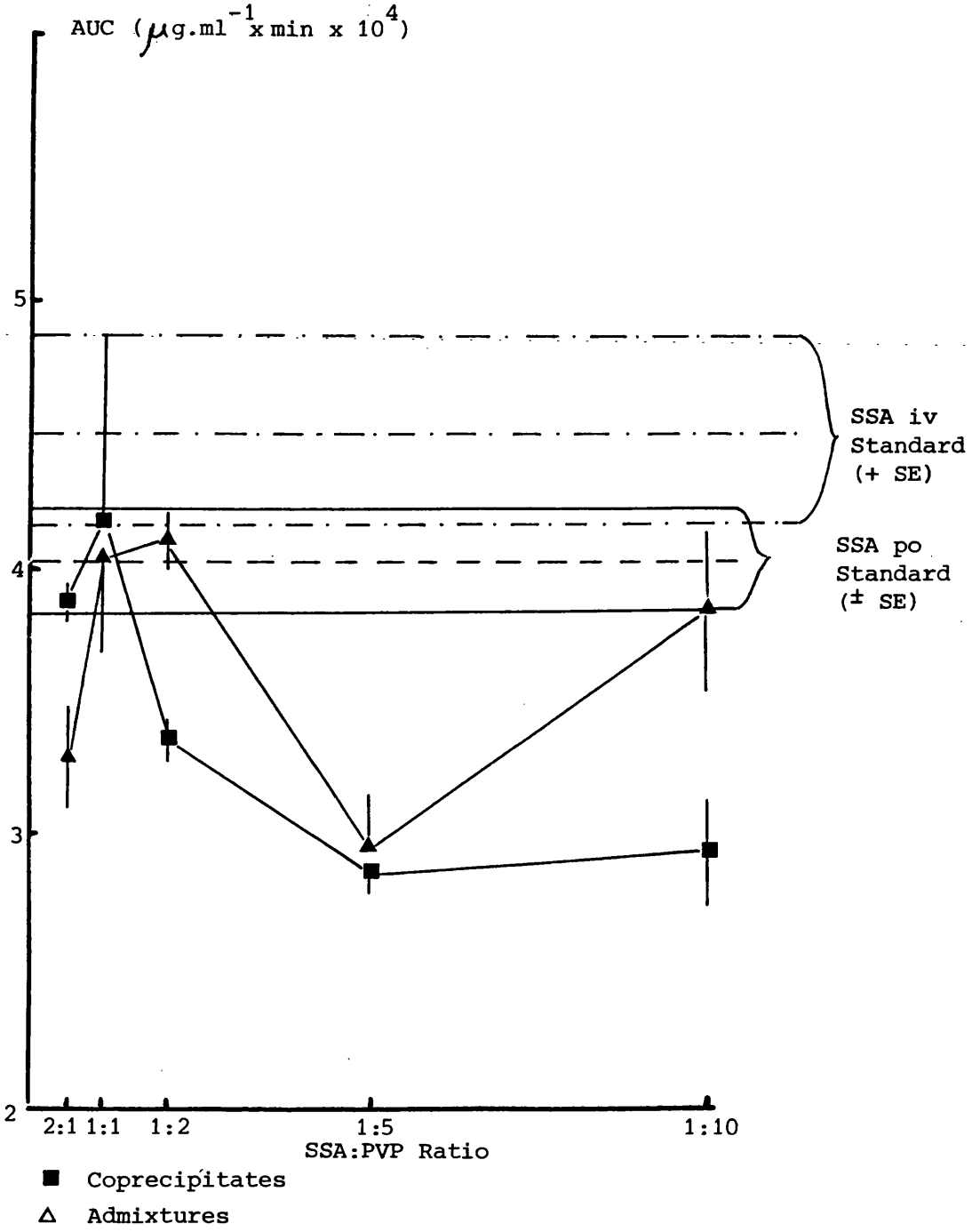


Fig. 4.3 Relation Between AUC And SSA:PVP Ratio For SSA:PVP Coprecipitates And Admixtures

Examination of the plasma salicylate peak times and concentrations (Fig. 4.4) provides a confusing analysis of bioavailability from SSA:PVP systems and it can be expected that other factors are influential. These factors include the effect of vehicle viscosity on drug dissolution, or the influence of both viscosity and osmotic pressure within the microenvironment of the absorptive membrane and on gastro-intestinal transit time. For the admixtures there are no obvious apparent changes that could be related to with SSA:PVP ratio other than the time required to attain the peak concentration (Fig. 4.4.B) and this may also be due to changes in gastric emptying rate etc, especially for the 1:2, 1:5 and 1:10 ratios with a proportionately higher PVP content.

The general conclusion from the in vivo absorption studies with SSA:PVP systems are that the amorphous nature of the SSA within the coprecipitates did not aid its absorption as might be expected on theoretical grounds. Coprecipitates and admixtures with PVP may, nevertheless, prove beneficial in SSA therapy by providing a method for delaying absorption in an attempt to prolong its anti-inflammatory/anti-rheumatic action, although the potential shown in the rat may not necessarily be confirmed in man.

4.12 SSA BIOAVAILABILITY FROM ITS COPRECIPITATES AND ADMIXTURES WITH CHOLIC ACID (CA) AND DEOXYCHOLIC ACID (DOCA)

The CA and DOCA systems are discussed as one section since the explanation offered for changes in bioavailability are applicable to both. Furthermore the gastro-intestinal

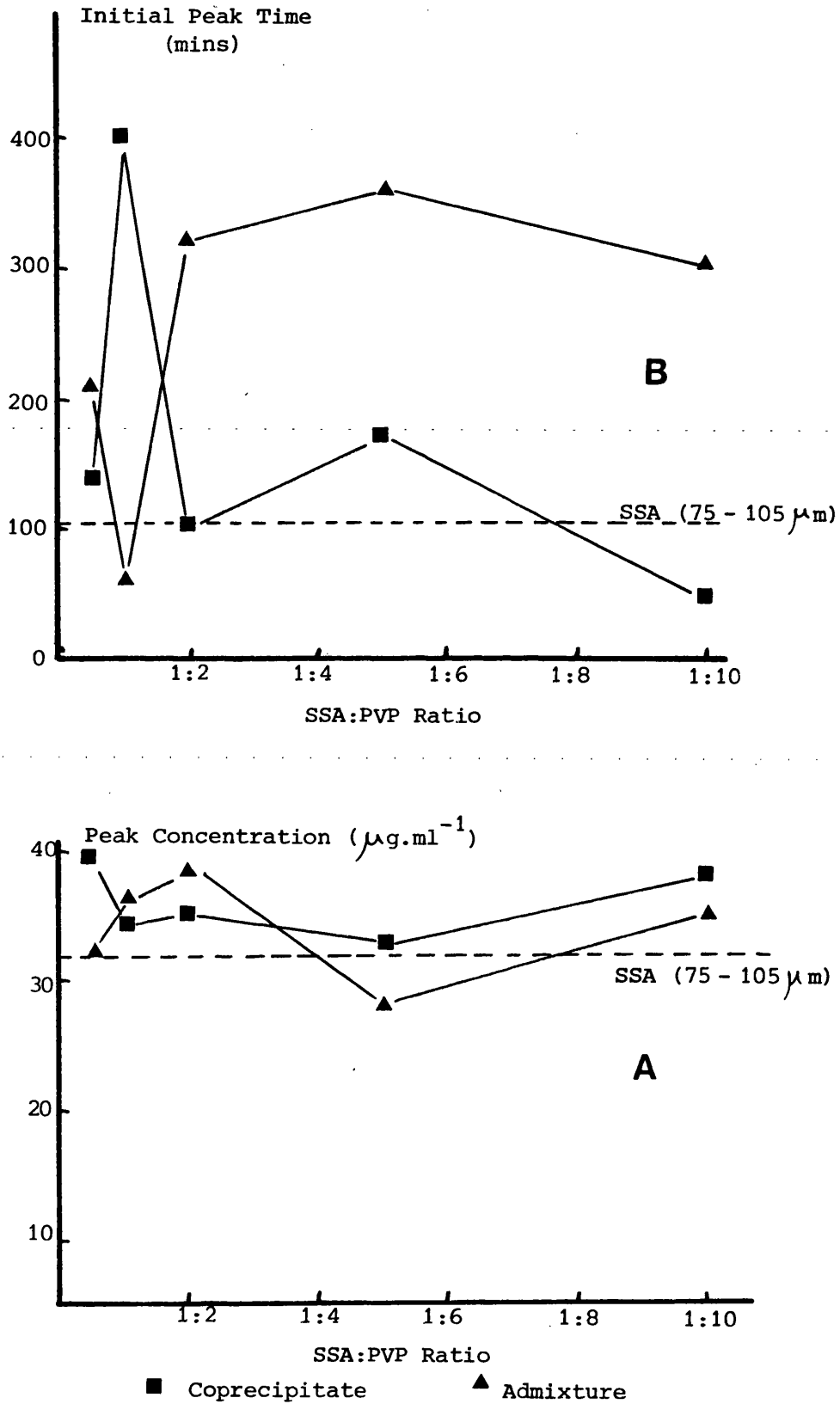


Fig. 4.4 Peak Plasma Salicylate Concentrations (A) And Times (B) For SSA:PVP Coprecipitates And Admixtures

disturbances observed for these bile acids will be contrasted and discussed.

Absorption of SSA from both the SSA:CA 1:1 ratio coprecipitate and admixture was markedly better than that for an equivalent oral dose of SSA alone and for all other SSA:CA systems. (Table 3.3.36). The area under the plasma salicylate profile for each has been given in Tables 3.3.33 and 3.3.34 for the coprecipitates and admixtures respectively and are further graphically expressed in Fig. 4.5.A. The AUC values for these 1:1 ratios are not significantly different from those for an equivalent intravenous dose. For 1:1 coprecipitate $t_{obs} = 1.69$; $N = 11$; $p' = 0.05$; $t_{tab} = 2.26$: For the 1:1 admixture $t_{obs} = 1.96$; $N = 10$; $p' = 0.05$; $t_{tab} = 2.31$. The influence of SSA:CA formulation on the plasma salicylate peak concentrations are shown in Fig. 4.5.B and can be seen to follow the same profile as for the respective AUC plot (Fig. 4.5.A). This similarity suggests that the enhanced bioavailability results from an increase in the dissolution rates of the 1:1 systems over those for the other SSA:CA systems and SSA alone. This is supported by the dissolution behaviour of the SSA:CA systems (Fig. 2.13.11) in which both 1:1 formulations show an enhanced rate of dissolution over those for the other SSA:CA ratios and the pure drug.

Bioavailability for the SSA:CA systems is given in Table 4.6 where the % available is calculated using AUC data with respect to the intravenous standard (Table 3.3.6).

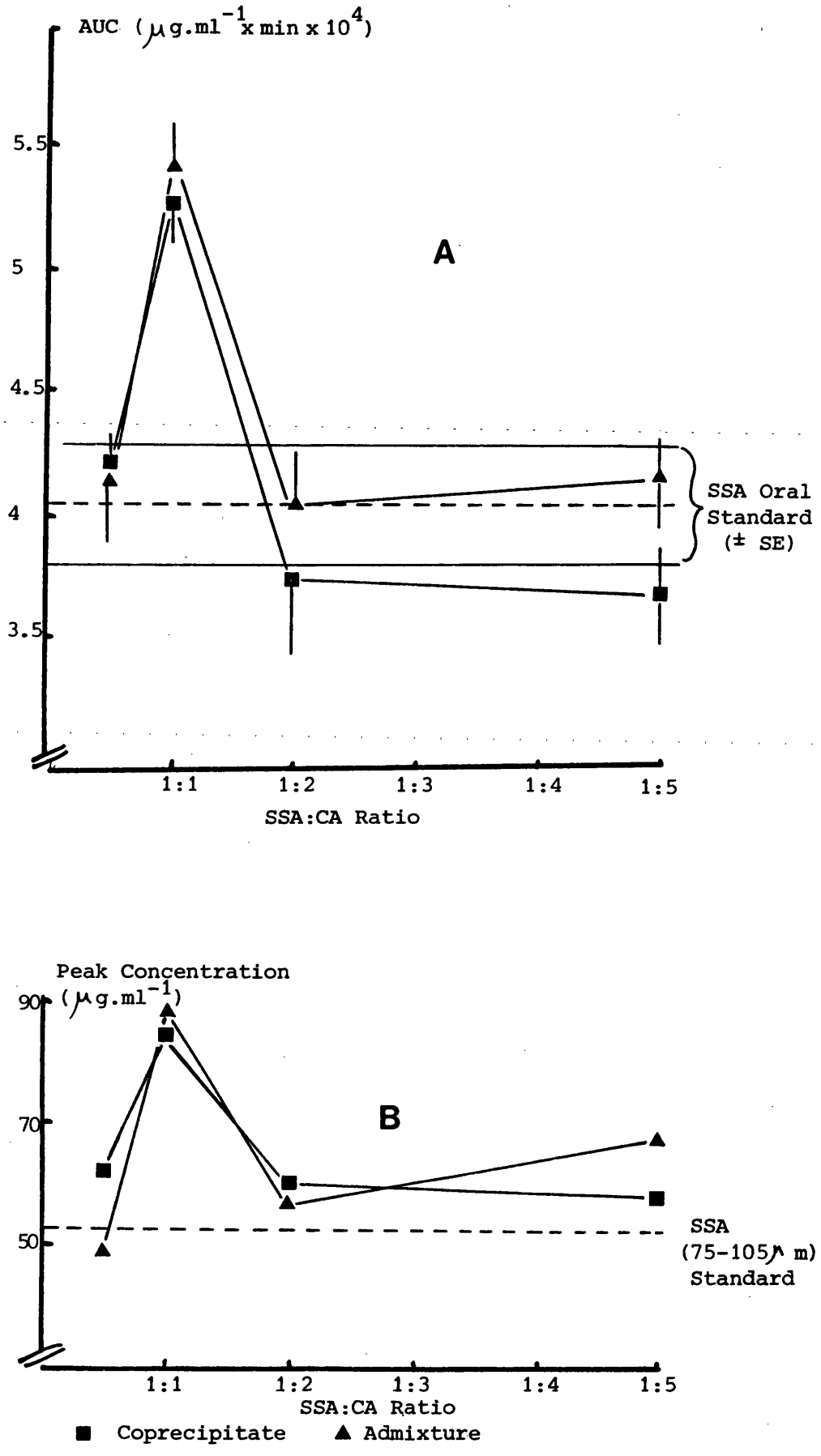


Fig. 4.5 Relationship Between AUC (A) And Peak Concentration (B) With SSA:CA Coprecipitates And Admixtures

Bioavailability (%)

SSA:CA ratio	SSA:CA COPRECIPITATES		SSA:CA ADMIXTURES	
	Mean AUC ($\mu g.ml.^{-1}xmin$)	% Bioav.	Mean AUC ($\mu g.ml.^{-1}xmin$)	% Bioav.
2:1	42133	93	41773	92
1:1	52695	116	54087	119
1:2	37352	82	40394	89
1:5	36775	81	41452	91

AUC reference iv 45383 $\mu g.ml.^{-1}xmin$ (From Table 3.3.6)

Table 4.6 Bioavailability For SSA:CA Coprecipitates And Admixtures Calculated From Mean AUC Data.

Cholic and deoxycholic acids are poorly water soluble and it can be assumed that in all orally dosed suspensions these bile acids were present in excess of their saturation solubility. Any solubilizing properties will therefore equally occur for each formulation; indeed the saturation solubility of SSA shows no difference between any of the SSA:CA ratios whereas each bile acid containing system showed a marginal increase in SSA solubility over that of the pure drug. (See Table 2.13.6). Similarly the wetting properties of cholic acid on the permeability of the gastric mucosae, although known to influence drug absorption (Bates et al, 1966; Gibaldi and Feldman, 1970;

among others), will be the same for each system and could not account for the increased bioavailability.

The influence of drug coprecipitation with bile acid systems on subsequent absorption has been suggested to be mediated through an increased surface area of the drug (Malone et al, 1966; Decato et al, 1969; Stoll et al, 1973): that is to say, during the coprecipitation process the bile acid provided a large surface area upon which the drug precipitates in a very finely-divided form. If during coprecipitation of the 1:1 ratio SSA and cholic acid combined within the organic solvent for from a 1:1 molar ratio molecular complex, then on removal of the solvent the resultant precipitate may have a differing crystal structure than the two original components. Such an occurrence, although not substantiated, may account for the unique amorphous nature of the SSA:CA 1:1 coprecipitate (Fig. 2.13.6). This does not, however, account for the improved absorption manifested by the 1:1 ratio admixture and it must therefore be assumed that SSA and cholic acid form a molecular complex in solution prior to absorption.

Absorption studies with the SSA:DOCA systems were marred by severe gastro-intestinal disturbance; consequently the majority of the coprecipitates and admixtures, although prepared and characterised, could not be evaluated in vivo. However the studies on the 2:1 and 1:1 systems that were performed show that, apart from the 2:1 coprecipitate, all other DOCA-containing formulations produced a significant reduction in SSA absorption in comparison to SSA alone (See Table 3.3.42 and

3.3.43). The bioavailability of salicylate from the SSA:DOCA formulations is given for each system in Table 4.7; the data was calculated with reference to an equivalent intravenous dose using AUC values.

	Bioavailability (%)			
	SSA:DOCA COPRECIPITATES		SSA:DOCA ADMIXTURES	
	Mean AUC ($\mu g.ml.^{-1}xmin$)	% Bioav.	Mean AUC ($\mu g.ml.^{-1}xmin$)	% Bioav.
2:1	45786	100	31668	70
1:1	28118	62	25963	57

AUC reference iv 45383 ($\mu g.ml.^{-1}xmin$) (From Table 3.3.6)

Table 4.7 Bioavailability For SSA:DOCA Coprecipitates And Admixtures Calculated From Mean AUC Data.

The poor bioavailability may be a consequence of several exacerbating properties of the bile acid, which include the solubilization of SSA within a micellar structure, drug:bile acid complexation, structural and functional deterioration of the gastro-intestinal mucosae and diarrhoea. Gastro-intestinal disturbance due to feeding non-physiological amounts of DOCA to rats has induced the copious production of liquid light-brown stools and ultimately resulted in death. (Shiner, 1969). The liquid nature of the stools results from reduced electrolyte

and water reabsorption from the lower sections of the intestine and is more acute following administration of deoxycholic than cholic acid (Mekhjian and Phillips, 1970; Gracey et al, 1973).

The reduced absorption for the SSA:DOCA systems is probably due to a combination of increased gastro-intestinal transit and impairment of the integrity of the mucosal epithelia (especially the villi and microvilli). Moreover, dissolution of nitrofurantoin:deoxycholic acid coprecipitates was faster in media at pH 7.4 than at pH 1.0 (Stoll et al, 1973). This was attributed to poor DOCA solubility, and could explain the lack of an early rise in plasma salicylate levels (before 100 minutes) and of secondary peaks since SSA:DOCA coprecipitate dissolution in the gastric environment would be small in comparison to that in the more alkaline small intestine. This would not account for the poor absorption for the SSA:DOCA admixtures and it must be assumed that the major influences of DOCA on reducing SSA absorption are those of alteration of the absorptive properties of the alimentary mucosae, complexation and/or increased gut transit associated with diarrhoea. This last point is exemplified by the reduced ampicillin absorption in subjects suffering with acute shigellosis (Nelson et al, 1972).

It should also be restated that the AUC values calculated for the DOCA containing systems may be exaggerated due to reductions in the apparent first order elimination rate constant as already discussed (See 4.7.2); the changes in AUC will therefore reflect the elimination as well as any perturbations in absorption.

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

The studies reported in this thesis have shown SSA to be poorly soluble and to exhibit dissolution rate-limited absorption. The *in vitro* studies pertaining to physical and chemical characterisation of factors influential on its absorption have provided information from which it may be concluded that, provided the drug is in solution then its absorption will probably occur throughout the entire length of the gastro-intestinal tract.

Examination of the bioavailability of SSA from various formulations was then performed using the measurement of total salicylate in body fluids. The major drawback from such an experimental approach has been the inability to describe the compartmental distribution of the drug from plasma level data by using the commonly employed techniques of 'feathering' and subsequent computerised analysis for the elucidation of the microconstants associated with absorption, distribution and elimination. From a retrospective overview of the *in vivo* studies it is readily apparent that more meaningful information regarding SSA bioavailability, absorption rates, etc would have been forthcoming had the time been spent in the development of a specific assay for SSA and its metabolites in body fluids. The most obvious techniques for this are those of gas-liquid chromatography and high pressure liquid chromatography. The latter offers the greater potential since it would not require the preparation of volatile derivatives, although the former would be able to provide a means of identifying metabolites etc if linked to a mass spectrometer. Furthermore, the saliva/plasma correlation studies would have undoubtedly benefited from a specific

assay for SSA, and with such an analytical tool it would have been possible to investigate first pass effects on SSA metabolism using *in situ* perfused intestine studies.

The preparation and *in vivo* evaluation of SSA coprecipitates with PVP, CA and DOCA has demonstrated that this type of formulatory manoeuvre does not necessarily result in an enhanced absorption and that such techniques may show potential for the control of drug release over a prolonged time period. The influences of drug coprecipitation would be better examined and compared to their physical mixtures by *in vitro* studies designed to monitor the release of both components from compressed discs of constant surface area. However, in the absence of the necessary apparatus and expertise for this type of study it should be put forward as a suggestion for further work, that would provide more information on the mechanisms of dissolution from experimental formulations than that obtained from studies on powders.

The work with PVP leaves open the possibility that other polymers may provide greater potential for altering the dissolution rate-limited absorption of some drugs since there must be many water soluble polymeric systems worthy of investigation.

The use of bile acids as a means of promoting drug bioavailability must be equated with the very real hazard of gastro-intestinal dyscrasia resulting in diarrhoea. In view of this the potential of such systems, although demonstrated *in vitro* and *in vivo* for SSA and nitrofurantoin (Stoll et al, 1973), must be limited if the drug is to be incorporated into formulations that are designed for chronic use.

Perhaps the use of inert, non-absorbable 'carrier systems', such as fumed silica dioxide, as suggested by Monkhouse and Lach (1972a) would also warrant further study.

APPENDICES

APPENDIX I

STATISTICAL ANALYSES

1. LEAST-SQUARES REGRESSION ANALYSIS

When a linear relationship is assumed to exist between variables it is usual to fit a straight line by least-squares regression analysis. The simplest statistical model for this assumes that the independant variable X is known without error of measurement, and that the corresponding measured values of the dependant variable Y are scattered normally from their time values. Hence each value Yi of the dependant is normally distributed about a mean.

The method of least squares obtains estimates of a and b in the equation $Y = x + bX$ such that the sum of the squares of the deviations of the observations Yi from their mean of $\alpha + \beta Xi$ is a minimum.

These values are:-

$$a = \frac{\Sigma Yi - b \Sigma Xi}{n} = \bar{Y} - b\bar{X}$$

$$b = \frac{n\Sigma XiYi - \Sigma Xi \Sigma Yi}{n\Sigma Xi^2 - (\Sigma Xi)^2}$$

$$= \frac{\Sigma (Xi - \bar{X}) \quad (Yi - \bar{Y})}{\Sigma (Xi - \bar{X})^2}$$

when n is the number of points defining the line.

1,a Variance of slope (b)

This is termed Sb^2 and is given by the equation:-

$$Sb^2 = \frac{\sigma e^2}{\Sigma (Xi - \bar{X})^2}$$

where σe^2 is the residual sum of variance of the dependant variable Y and is obtained from

$$\sigma e^2 = \frac{\Sigma D^2}{(n-2)}$$

where ΣD^2 is the residual sum of squares.

ΣD^2 is obtained from the equation

$$\Sigma D^2 = \Sigma (Yi - \bar{Y})^2 - b^2 \Sigma (Xi - \bar{X})^2$$

The standard error of the slope is given by the square root of the variance.

1,b Variance of the Intercept (a)

This is termed $Sa^2 = \frac{\Sigma Xi^2 \sigma e^2}{n \Sigma (Xi - \bar{X})^2}$

where $\sigma e^2 = \frac{\Sigma D^2}{(n-2)}$

The standard error of the intercept is given by the square root of the variance.

1,c Correlation Coefficient

The correlation coefficient (r) is defined as:-

$$r = \frac{\Sigma (Xi - \bar{X}) \quad (Yi - \bar{Y})}{\sqrt{\Sigma (Xi - \bar{X})^2 \quad (Yi - \bar{Y})^2}}$$

To represent a linear relationship between two variables, X and Y, r must be $\pm$ unity. The calculated value of r is compared with the tabulated value at the 5% probability level for n - 2 degrees of freedom, and, if found to be greater than the tabulated value, the observations were considered to be linearly related.

2. TO DETERMINE THE EQUALITY OF TWO ESTIMATES OF A PARAMETER

The test is generally known as the Student 't' test. The equality of estimates P_1 and P_2 with variances S_1^2 and S_2^2 respectively of a parameter P is assessed by means of the following statistic:-

$$t = \frac{P_1 - P_2}{\sqrt{S_1^2 + S_2^2}}$$

The value of 't' is compared with tabulated values with $n_1 + n_2 - 4$ degrees of freedom where n_1 and n_2 are the number of observations used in the estimation of P_1 and P_2 respectively. If the calculated value of 't' exceeds the tabulated value at the 5% probability level, the parameters are considered to be significantly different at that level.

3. TO DETERMINE THE EQUALITY OF MORE THAN TWO ESTIMATES OF A PARAMETER

This is generally known as the Bartlett Test. When more than one estimates of a parameter P are tested for equality the following statistic is used:-

$$B = \frac{\Sigma (Pi - \bar{P})^2}{\hat{\sigma}^2}$$

where $\hat{\sigma}^2$ is given by

$$\hat{\sigma}^2 = \frac{n_1 S_1^2 + n_2 S_2^2 + \ldots\ldots\ldots\ldots\ldots\ldots n_n S_n^2}{n_1 + n_2 + \ldots\ldots\ldots\ldots\ldots\ldots n_n}$$

where S_1 and S_2 etc are the standard errors associated with the estimates P_1, P_2, etc and, n_1 and n_2 are the number of observations used in determining the estimates. If the estimates all come from the same normal distribution, $\frac{\Sigma (Pi - \bar{P})^2}{\hat{\sigma}^2}$ will have a χ^2 distribution with N - 1 degrees of freedom, where N is the number of estimates.

4. ANALYSIS OF VARIANCE

The contribution to the total variance of a process that is made by the variance of each individual component of that process can be determined by an Analysis of Variance. Where data can be grouped as a series of columns each consisting of a number of samples, the analysis of variance enables computation of the variance attributable to the differences between columns (σA^2) and the variance attributable to the differences within columns

(σ_B^2). This analysis of variance can be calculated as follows, and the data tabulated as indicated.

1. Square the individuals and add
2. Obtain the total for each column, square these totals, sum these squares and divide the total by the number of individuals in each column
3. Obtain the grand total for all individuals, square this grand total and divide by the grand total number of individuals.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	Components of Variance
Between Columns	2 - 3	m - 1	$\frac{2 - 3}{m - 1}$	$n\sigma_A^2 + n\sigma_B^2$
Within Columns	1 - 2	mn - m	$\frac{1 - 2}{mn - m}$	σ_B^2
Total	1 - 3	mn - 1		

Where m is the number of columns

n is the number of individuals in each column

σ_A^2 is the variance due to differences between columns

σ_B^2 is the variance within columns

To test if the between column variance is significantly greater than the within column variance, the ratio (F) of the between column mean square of the within column mean square is determined.

If this value is less than the tabulated value at $p' = 0.05$ with $m - 1$ and $mn - m$ degrees of freedom, then it follows that the variance due to differences between columns is not significant.

Analysis of variance was carried out using a computerised system that allowed the analysis of data that did not form a complete matrix (Nedler, 1973). Missing values were estimated by the programme and incorporated into the calculation of the variance. All calculations were performed on an ICL system 4 computer at the University of Bath, Computer Centre, and the author is most grateful to Mr D. G. D. Clark for his considerable assistance.

All tabulated values for the Student 't' test, correlation coefficient, χ^2 and analysis of variance were obtained from Fisher and Yates (1974).

APPENDIX II

The determination of ionisation constants by ultra-violet spectrophotometry depends on the ionic species of the compound exhibiting different absorption spectra. If this criterion is satisfied a wavelength is chosen at which the greatest difference between the absorbances of the species is observed. Measurement of absorbance at this 'analytical wavelength', at pH values intermediate between those at which only one or other of the two species is present, enables the ratio of the species to be calculated. Two basic assumptions are made in this calculation: firstly, that the total drug concentration remains constant, and secondly, that both species of the drug obey the Beer-Lambert law. Under such conditions the resultant absorbance (A) will be due to the sum of the absorbances of the ionised (A_I) and unionised (A_u) species.

$$\text{i.e.} \quad A = A_I + A_u \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (50)$$

The absorbance of the unionised species is given by:-

$$A_u = \epsilon_u . c . \ell \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (51)$$

and for the ionised species

$$A_I = \epsilon_I . c . \ell \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (52)$$

where ϵ_u and ϵ_I are the molar absorbance coefficients for the unionised and ionised species respectively; C is their concentration and ℓ the pathlength (taken as 1 cm). Therefore in a mixture of both

species at a known pH the absorbance at that pH (A_{pH}) is given by:-

$$A_{pH} = A_u f_u + A_I f_I \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (53)$$

where f is the fraction unionised or ionised respectively. For the ionised species alone the absorbance at the 'analytical wavelength' at any measured pH is given by:-

$$A_{pH} = \epsilon_u (1 - f_I) + \epsilon_I f_I \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (54)$$

$$= \epsilon_u - \epsilon_u f_I + \epsilon_I f_I$$

$$= \epsilon_u + f_I (\epsilon_I - \epsilon_u)$$

$$f_I = \frac{A_{pH} - \epsilon_u}{\epsilon_I - \epsilon_u} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (55)$$

Similarly for the unionised alone:-

$$A_{pH} = \epsilon_I (1 - f_u) + \epsilon_u f_u \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (56)$$

$$= \epsilon_I - \epsilon_I f_u + \epsilon_u f_u$$

$$= \epsilon_I + fu (\epsilon_u - \epsilon_I)$$

$$f_u = \frac{A_{pH} - \epsilon_I}{\epsilon_u - \epsilon_I} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (57)$$

which can be rewritten as:-

$$f_u = \frac{\epsilon_I - A_{pH}}{\epsilon_I - \epsilon_u} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (58)$$

By substitution of the respective fractions for concentrations in the Henderson-Hasselbalch equation (8) we get:-

$$pH = pKa + \log \frac{f_I}{f_u} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (59)$$

and by further substitutions for f_I and f_u and rearrangement:-

$$pH = pKa + \log \frac{A_{pH} - \epsilon_u}{\epsilon_I - A_{pH}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (60)$$

Acceptance of the basic assumptions of constant concentration and the adherence of both ionic species to the Beer-Lambert law then:-

$$\epsilon_u \propto A_u \quad \text{and} \quad \epsilon_I \propto A_I$$

$$\text{Therefore} \quad pH = pKa + \log \frac{A_{pH} - A_u}{A_I - A_{pH}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (61)$$

$$\text{or} \quad pKa = pH + \log \frac{A_I - A_{pH}}{A_{pH} - A_u} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (62)$$

If $A_{pH} \gg A_I$ equation (62) can be rearranged thus:-

$$pKa = pH + \log \frac{A_{pH} - A_I}{A_u - A_{pH}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (63)$$

Therefore for a constant concentration the pKa for any ionisation can be calculated from the absorbance at a measured pH and knowledge of the absorbance for the drug in the totally unionised and ionised forms.

APPENDIX III

Calculation of the Area Under the Curve (AUC) Using the Trapezoidal Rule.

AUC calculations were made using the program given overleaf. The program is written in ALGOL and receives raw data from which it determines the sum of all trapezoids described by the experimental data points provided.

```
BEGIN
INTEGER N,X,J;
INTEGER Z;
REAL AUC,TRI1,TRI2,SUMTRAP;
REAL ARRAY TRAP(0::50);
REAL ARRAY Y,T,DELTAT(0::50);
START:
READ (X);
IF X=0 THEN GOTO STOP;
READ (N);
READ (Z);
FOR J:=1 STEP 1 UNTIL N DO
READ (T(J),Y(J));
FOR J:=N STEP -1 UNTIL 2 DO
BEGIN
DELTAT(J):=T(J)-T(J-1);
END;
FOR J:=1 STEP 1 UNTIL N-1 DO
BEGIN
TRAP(J):=((Y(J)+Y(J+1))/2)*DELTAT(J+1);
END;
SUMTRAP:=0.0;
FOR J:=1 STEP 1 UNTIL N-1 DO
BEGIN
SUMTRAP:=SUMTRAP+TRAP(J);
END;
AUC:=SUMTRAP;
IF X=1 THEN
BEGIN
WRITE("CALCULATION OF AREA UNDER PLASMA LEVEL CURVE");
END;
IF X=2 THEN
BEGIN
WRITE("CALCULATION OF AREA UNDER SALIVA LEVEL CURVE");
END;
IF X=3 THEN
BEGIN
WRITE("CALCULATION OF AREA UNDER URINARY EXCRETION CURVE");
END;
WRITE(" ");
WRITE(" ");
WRITE("                                        T(J)              Y(J)     ");
WRITE(" ");
FOR J:=1 STEP 1 UNTIL N DO
BEGIN
WRITE("                          ",T(J),Y(J),);
WRITE(" ");
END;
WRITE("AREA UNDER CURVE =", AUC,);
IF Z=1 THEN
WRITEON("MG/ML *HR");
IF Z=2 THEN
WRITEON("MG/ML *MIN");
IF Z=3 THEN
WRITEON("MICROGRAM/ML *HR");
IF Z=4 THEN
WRITEON("MICROGRAM/ML *MIN");
IF Z=5 THEN
WRITEON("UNITS/ML *HR");
IF Z=6 THEN
WRITEON("UNITS/ML *MIN");
IF Z=7 THEN
WRITEON("MG *HR");
IF Z=8 THEN
WRITEON("MG *MIN");
WRITE(" ");
GOTO START;
STOP: END.
%DATA
```

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